MATERNAL PLASMA 6-KETO PROSTAGLANDIN $F_{1\alpha}$ IN PREECLAMPSIA

Augusta Simpson Roth

1982

Permission for photocopying or microfilming of "Maternal Plasma 6-keto PGF1α in Preeclampsia"

(Title of thesis)

for the purpose of individual scholarly consultation or reference is hereby granted by the author. This permission is not to be interpreted as affecting publication of this work or otherwise placing it in the public domain, and the author reserves all rights of ownership guaranteed under common law protection of unpublished manuscripts.

Augusta Roth

Signature of Author

3/3/82

Date

MATERNAL PLASMA 6-KETO PROSTAGLANDIN $F_{1\alpha}$ IN PREECLAMPSIA

Augusta Simpson Roth
B.S.E.E.
University of Arizona

A Thesis Submitted to the Yale University School of Medicine in Partial Fulfillment of the Requirements for the Degree of Doctor of Medicine
1982

Abstract

The possibility of decreased prostacyclin (PGI_2) in preeclampsia-eclampsia is currently being studied by several laboritories. Because of prostacyclin's various actions (platelet aggregation inhibition,vasodilation, stimulation of renin release, and possible inhibition of the hypertensive effects of angiotensin II), an increase in the peripheral activity of this chemical could explain much of the altered physiology of normal pregnancy. A relative decrease in peripheral activity could tie together the various pathophysiological findings of preeclampsia-eclampsia.

Therefore, plasma 6-keto $PGF_1\alpha$, the stable nonenzymatic metabolite of prostacyclin, was measured in women with preeclampsia, and in those women of various gestational ages having a normal pregnancy. The detection method, radioimmunoassay, could not be internally validated. It appears that the major problem with the assay was in sample preparation.

Plasma 6-keto $PGF_1\alpha$ may be elevated in normal pregnancy. A decrease in plasma values was seen in those with preeclampsia as compared to normals of the same gestational age. In addition, plasma 6-keto $PGF_1\alpha$ was found to correlate with platelet count in the preeclamptic women.

The results of this study cannot be accepted as reliable, but instead should be accepted as impetus for further study. Gas-Chromatography-Mass Spectrometry (GC-MS) of some of the samples could be used for verification of this work. Platelet aggregation inhibition bioassay, along with confirmation by GC-MS, would be the best method for future study.

If PGI_2 activity and concentrations are found to truly be decreased, not only would tne mechansim of preeclampsia-eclampsia become more clear, but a more acceptable treatment for the disease would be suggested.

Acknowledgements

The help of many people made this project possible. I was very lucky to have two exceptionally interested and involved advisors - Drs. Harold R. Behrman and Roberto Romero. Dr. Romero, as clinical advisor, suggested the project, alerted me whenever possible participants were admitted, and was a constant source of enthusiasm. Dr. Behrman, as laboratory advisor, gave me space in his lab and access to materials and equipment. It was rare and enjoyable to find two professors who were always willing to find time to talk to a student.

The staff at the YNHH Women's Center and Labor floor were very helpful in alerting me to women who might be eligible for the study. Everyone in the Gynecology Endocrine Lab, especially Mark Linsky and Sandy Preston, showed a lot of patience in showing me techniques and sharing lab equipment.

And finally, Bruce Roth must be thanked for all of his help. Who else would be willing to help me set up an assay at 3:00 am, proofread the rough draft and final copy, and allow the living room to be covered with reference articles, all without a complaint?

Table of Contents

I. Preeclampsia-eclampsia

A. Clinical Definitions

Preeclampsia-eclampsia is a common disease of pregnancy, with an incidence of about 3% of all pregnancies. The etiology is unknown, despite decades of intensive research.

The change in definitions and classifications of hypertensive diseases of pregnancy reflects the realization that several disease processes with several etiologies are involved. The old term "toxemia of pregnancy" was formerly applied to any disorder involving hypertension, proteinuria, or edema in pregnancy or the puerperium. In 1972 the Committee on Terminology of the American College of Obstetricians and Gynecologists (1) devised the following classification:

preeclampsia Hypertension with proteinuria, edema, or both induced by pregnancy in the twentieth week of gestation or later, or before the twentieth week if there is hydatidiform change.

eclampsia Convulsion not caused by neurological disease in a woman who fulfills the criteria for preeclampsia.

superimposed preeclampsia-eclampsia Either disorder in a woman with chronic hypertensive vascular disease or kidney

disease.(Blood pressure must rise 30 mm Hg systolic or 15 mm Hg diastolic.)

chronic hypertensive disease Persistent elevation of blood pressure before the twentieth week or beyond six weeks postpartum.

gestational hypertension Hypertension developing in the last half of pregnancy or the first 24 hours postpartum, without proteinuria or edema.

gestational edema Generalized edema that persists after twelve hours of bedrest, or five or more pounds of weight gain in one week.

gestational proteinuria in pregnancy not induced by hypertension, infection, edema, or known vascular or renal disease.

hypertension Diastolic pressure of 90 mm Hg or more; or systolic pressure of 140 mm Hg or more; or a rise of diastolic pressure of 15 mmHg or more; or a rise of 30 mm Hg systolic or more which occurs on two separate occasions at least six hours apart.

proteinuria 0.3 g/l of protein in urine collected over a twenty four hour period.

edema weight gain of at least 1 kilogram in one week when accompanied by generalized edema.

Preeclampsia is defined as either mild or severe. It is severe if any of the following occur:

1) blood pressure greater than 160 systolic or 110 diastolic that does not decrease with hospitalization and bedrest.
2) at least 5 grams proteinuria in twenty-four hours, or 3 - 4 quantitative.
3) oliguria (less than 400 cc of urine per day)
4) cerebral or visual symptoms
5) epigastric or right upper quadrant pain.
6) pulmonary edema or cyanosis.
7) thrombocytopenia
8) hepatocellular damage.
9) fetal growth retardation.

Seven to ten percent of all pregnancies are complicated by hypertension. About half of these are due to chronic hypertension and half due to preeclampsia. (2) Despite its high incidence, studies of the disease in the past have been hampered by the lack of precise diagnoses and definitions. For example, when proteinuria and hypertension have been present before conception, they can often disappear during the third trimester, only to reappear in the third and continue their presence after delivery.(3) Thus differentiating between preeclampsia, chronic hypertensive disease, and superimposed preeclampsia can often be done only retrospectively.

B. Pathophysiology

1. Vascular Changes

Vasospasm is a basic component of preeclampsia. The hypertension of preeclampsia-eclampsia is due to widespread systemic arteriolar vasospasm (3,4,5), which causes increased peripheral resistance. Although evident through-

out the body, it is thought to be most significant in the kidneys and uterus. (6) The spasm is segmental, leading to areas of alternating dilation and contraction and intermittent interruption of capillary circulation.

The plasma volume in normal pregnancy is increased forty to fifty percent over normal nonpregnant levels. (7) This is only partially explained by the physiologic increase in aldosterone. In preeclampsia, the increase in plasma volume is less or not evident. In severe disease, the plasma volume may decrease as much as thirty to forty percent (8) despite an increase in total body water and in sodium retention. The smaller intravascular volume and space makes preeclamptic women much less tolerant to fluid overload or blood loss. (9)

Vascular sensitivity is increased in women who develop preeclampsia. In normal pregnancy, there is a two-fold increase in renin, RPA, angiotensin, and aldosterone (6,10,22, 11) and a fifty percent increase in cardiac output (12), but blood pressure is decreased. This occurs because a refractoriness to angiotensin II's hypertensive effects develops. This refractoriness is not related to blood volume or angiotensin II levels. (13) The increases in the renin-angiotensin-aldosterone system contribute to the physiologic hypervolemia while blood pressure remains low due to vascular insensitivity. Gant (14) showed that an increase in sensitivity to the hypertensive effect of angiotensin II

occurs in preeclampsia, and that this change antedates the onset of hypertension.

An increase in vascular sensitivity to other pressor hormones such as norepinephrine and vasopressin has been shown in preeclamptics.(15) In fact, a pressor response to lying supine after lying in the left lateral recumbent position has been shown in women who are destined to become hypertensive.(11) Thus it appears that there is a definite vascular hypersensitivity in women who will develop preeclampsia.

2. Endocrinological Changes

The increase in plasma renin,angiotensin II, and aldosterone that occurs normally in pregnancy is significantly blunted in preeclampsia. (6,10,11,16) Vasopressin (17), deoxycorticosterone (15), and progesterone (18) levels are not different from levels seen in normal pregnancy.

Renin is produced in both the uterus and the juxtaglomerular apparatus of the kidney. Part of the decrease in renin release may be due to the inhibitory effects of sodium retension and hypertension on the JGA.

Prostaglandins are known to help regulate the release of renin, at least in the kidney. Recent evidence (19,20) that one of the prostaglandins may be the final common pathway for the different signals (sodium depletion, hypotension, hemorrhage, dehydration, constriction of the renal artery or aorta) that increase renin output. (21) The most likely prostaglandin is prostacyclin. It is the major

product of arachidonic acid metabolism in the renal arterial tree, and prostacyclin is known to cause renal renin release *in vitro*. (12,19,3)

3) Uteroplacental Changes

It is commonly thought that placental perfusion is decreased in women with preeclampsia (15) although precise blood flow measurment techniques are not yet available for use in pregnant women. The rate of dehydroisoandrosterone sulfate (DS) being converted into estradiol 17β by the placenta has been shown to reflect placental blood flow. (23,24) Normally, this conversion increases steadily throughout pregnancy. In women who later develop hypertension, the conversion at first is greater than in normals, and then drops as hypertension develops. The excessive clearance levels antedating preeclampsia may correlate not with increased placental blood flow, but with "hyperplacentosis," an excess of functioning placenta. Preeclampsia occurs with higher frequency in those with hydatidiform mole, or in maternal diabetes, erythroblastosis fetalis, and multiple gestation, all conditions in which there are large placentas. (15)

A definite morphological difference can be seen in the placental bed of women with preeclampsia. A lack of "physiological changes" in the uterine spiral arteries of preeclamptics has been shown by several investigators. (25,

26,27) In normal preganacy, the spiral arteries dilate from the distal end proximally into the myometrial segments figure 1).(27)

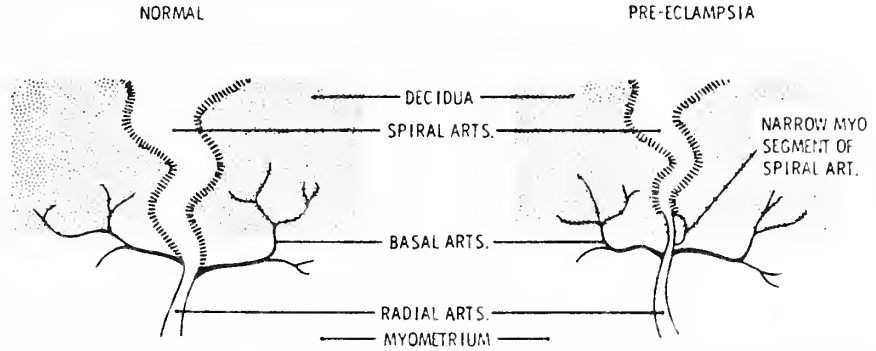


fig. 1. Spiral arteries in normal and preeclamptic pregnancies. From (27)

This is caused by migrating endovascular trophoblast cells replacing the reactive musculoelastic tissue that lines the arteries. Such tissue infiltrates in two waves; one into the decidua, then after a four to six week time lag,into the myometrail spiral arteries. In preeclamptics, the changes are restricted to the decidual segments. Some phenomenon appears to inhibit the second wave on endovascular trophoblastic infiltration, resulting in a relatively narrowed deciduomyometrial junction and the continued presence of reactive musculoelastic cells surrounding the arteries.

With the onset of clinical preeclampsia, another placental vascular lesion occurs. Focal areas of necrosis through the vascular walls develops. This "acute atherosis" (25, 26) is seen only in the small uterine vessels. Elecronmicroscopic evidence shows early accumulation of lipid in the myointimal cells, and necrosis of medial smooth muscle. This

later advances to lesions with numerous macrophages present and with fibrin deposition. (27)

4) Disseminated Intravascular Coagulation

During uncomplicated pregnancy, there is an increase in many of the factors of coagulation, such as fibrinogen, prothrombin, factors VIII, VII, IX, and X, soluble fibrin, and fibrin degradation products. (12,28,29,30) Fibrinolysin is slightly decreased, as is the platelet count.

With preeclampsia, there is greater coaguability and a decrease in blood volume. (28) In the hypercoaguability of preeclampsia, changes are in the same direction as in normal pregnancy, but of a greater degree. This degree reaches the level of manifest overcompensated disseminated intravascular coagulation (DIC), with severe thrombocytopenia, high fibrinogen, and disseminated intravascular obstructions, in about 3% of preeclamptics and 9% of eclamptics. (28) About 15% of all preeclamptics develop some degree of thrombocytopenia.

Coagulation parameters can be used to monitor the severity of the disease. Platelet count is lower and platelet lifespan is shorter in preeclamptics (5.1 days) as compared to normals (8.9 days) and nonpregnants (9.1 days). (31) This is due to increased platelet consumption and microthrombi formation, which may be responsible for peripheral organ damage. Platelet count, platelet serotonin levels,

fibrin degradation products, and Factor VIII concentrations have been shown to parallel the severity of preeclampsia. (30,32) Thus the hypercoaguability of preeclampsia appears to be part of a continuum between the low level chronic intravascular coagulation of normal pregnancy, and DIC seen in a subgroup of preeclamptics and eclamptics. This concept agrees with the commonly held idea that coagulation changes in preeclampsia are probably secondary to a more basic process.

5. Renal Changes

In normal pregnancy, glomerular filtration rate (GFR) and renal blood flow (RBF) are increased over nonpregnant values. This situation is reversed in preeclampsia. As the disease becomes more severe, there is a progressive decrease in renal function (lowered GFR and lower clearances of inulin,uric acid,creatinine) along with decreased renal perfusion. (4,15,33) The proteinuria seen in the disease is a nonselective middle molecular weight proteinuria consistent with functional glomerular lesions. (33,34) One study suggested that there might be two forms of proteinuria; one due to the vasoconstrictor effect of hypertension (with relatively good fetal prognosis) and one due to membraneous changes (with poorer fetal prognosis). (35)

Histologic changes found in kidney biopsies and at autopsies are common. At the present time, there is de-

bate over the existence of a pathognomonic kidney lesion. Glomeruli are usually enlarged with dilated capillary loops, foamy swelling of the endothelial cells, and subendothelial deposits of protein, together called glomerular capillary endotheliosis. (15,36) The lesion has never been found in nonpregnant women or in men. Fibrin/fibrinogen, immunoglobulins (IgG and IgM) and complement deposition are less constant features. On rare occasions, renal cortical necrosis occurs secondary to spasm of the renal arteries. No morphologic changes in the renal arterioles have been shown. The presence and duration of proteinuria corresponds to the occurance and severity of renal lesions. (37)

6. Hepatic Changes

Periportal hemorrhagic necrosis was considered formerly to be a characteristic of eclampsia. This lesion was usually found in fatal cases that went to autopsy. In liver biopsies done on six preeclamptics and twelve eclamptics, the liver biopsies appeared normal. (15) Liver function tests are rarely abnormal in the disease; SGOT and SGPT are occasionally raised, and bilrubin is rarely elevated, although one report noted SGOT and SGPT elevations in eclamptics but not preeclamptics. (38) Most investigators now consider the pathological changes in the liver to be variable, and most likely to be secondary to the more basic vascular/coagulation abnormalities seen in the disease.

7. Cardiopulmonary Changes

The heart is subjected to the hemodynamic stresses of decreased intravascular volume and increased peripheral resistance in preeclampsia. Heart failure from circulatory overload (due to overly vigorous intravenous fluid administration without correction of vasospasm) has occured in some cases. (15,39) Pulmonary edema is often found at autopsy. Cardiorespiratory failure, along with cerebral hemorrhage, account for most of the maternal deaths occurring in preeclampsia-eclampsia. It appears that these events are not part of the primary disease process, but occur from overtaxing the cardiovascular system with severe hemodynamic stresses.

Pulmonary arteriolar resistance has been measured by Swan-Ganz catheter in three severe preeclamptics, and was shown to be at or below nonpregnant levels. (5) Although no data is available for arteriolar resistance in normal pregnant women, this finding indicates that the pulmonary vasculature is not involved in the vasospasm seen throughout the systemic circulation.

8. Cerebral Changes

The major brain lesions found at autopsy in women who died from eclampsia are edema, hyperemia, thrombosis, and hemorrhage, which varies from petichiae to gross hemorrhage. Cerebral bleeding is one of the major causes of death. Hem-

orrhagic lesions are often found in the brains of women who died of other causes. (15)

Nonspecific electroencephalographic changes usually exist for some time after the eclamptic seizures. Coma for up to 3 days was reported in six of a series of fifty-two preeclamptics. (39) The cause of eclamptic seizures and their link to the pathological changes in preeclampsia are still unknown. Some young women may have seizures with a blood pressure of only 145/85, while others can withstand pressures of 180/120 without convulsions.

II. Prostaglandins and Preeclampsia-eclampsia

A. Metabolites and Actions

The metabolites of arachidonic acid form a group of very potent compounds with a variety of vasoactive and other effects. They are present in nearly every tissue studied to date.

Precursors are stored as $C_{20:4}$ fatty acid esters within cell membranes. (42) Stimuli such as hormones, antigen challenge, collagen, and thrombin stimulate the release of fatty acids (mostly archidonic acid) by a number of acyl-hydrolases such as phospholipase A_2 and triglyceride lipase. Platelet release of arachidonic acid may involve several enzymes such as phospholipase C and diglyceride lipase. (43)

Once arachidonic acid is released, it is quickly metabolized by one of two different pathways. It can be converted by by fatty acid lipoxygenase into the hydroxyacids HPETE (15-hydroxy-5,8,11,13-eicosatetraenoic acid) and HETE (hydroxyeicosatetraenoic acid), and to the leukotrienes. The required enzyme for this pathway has so far only been found in platelets, white cells, and lung tissue. The more common pathway involves conversion by fatty acid cyclooxygenase (also called prostaglandin endoperoxide synthetase) into

the 15-hydroxy prostaglandin endoperoxides PGG_2 and PGH_2. (43,44,45) PGG_2 and PGH_2, with half lives of about five minutes at 37 C, are the intermediates in the synthesis of prostaglandins, thromboxanes, and prostacyclin. (fig. 2) Agents that are known to inhibit the various steps of arachidonic acid metabolism are shown below. (44,46)

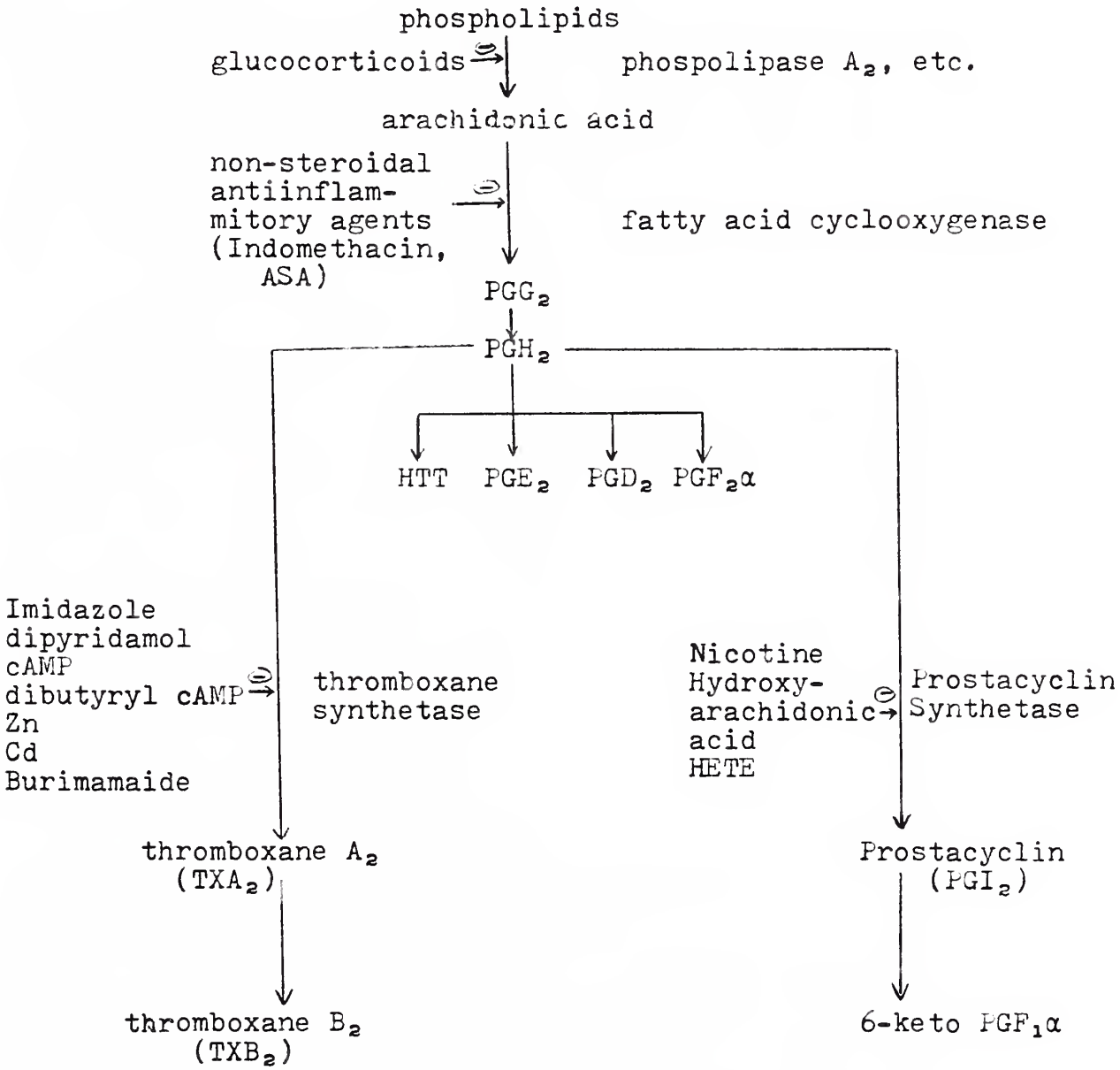


Fig. 2. Arachidonic Acid Metabolism.

Two of the most studied products are the vasoactive chemicals thromboxane A_2 (TXA_2) and prostacyclin (PGI_2), which act as natural antagonists. TXA_2 is made chiefly by the platelets, and is a potent vasoconstrictor and stimulator of platelet aggregation. Prostacyclin, made by the vascular endothelium and other tissues, is a potent vasodilator and platelet aggregation inhibitor. An extensive research effort surrounding these two products is being conducted because it is thought that an inbalance between these two could be a final common pathway in the development of essential hypertension and cardiovascular degeneration.

1) Thromboxane A_2

The chemical structure of TXA_2 was first described in 1975. (47) TXA_2 causes irreversible platelet aggregation and platelet release of ADP, serotonin, platelet factor IV, calcium, lysosomal enzymes, HETE (an inhibitor of prostacyclin synthetase), a smooth muscle growth stimulating substance, and more TXA_2. (45) TXA_2 also causes vascular smooth muscle contraction.

PGH_2 is converted to TXA_2 by thromboxane synthetase, an enxyme found in platelets and the microsomal fraction of tissue homogenates. (44) Originally called Rat Aorta Contracting Substance, TXA_2 has a half life of 32 minutes at 37 C and pH 7.4. It breaks down spontaneously into thromboxane B_2 (TXB_2), which is a stable, inactive metabolite.

(48) No other metabolites have been reported.

Selective inhibition of TXA_2 synthesis would theoretically be important for the prevention of cardiovascular disease. Aspirin was initially thought to selectively block platelet cyclooxygenase as compared to vessel wall cyclooxygenase. (49) It is now apparent that aspirin binds irreversibly to cyclooxygenase in both tissues. However, circulating platelets lack the ability to synthesize new enzyme during its nine day life in the bloodstream. Endothelium is able to make new cyclooxygenase. (45) Thus a low dose ASA could be useful in increasing the ratio of prostacyclin/thromboxane. Clinical studies on aspirin's effectiveness are not complete.

A more direct approach would be to specifically block thromboxane synthetase. Several specific compounds, such as imidazole, dipyridamol, cAMP, dibutyryl cAMP, and burimamide have been found to be inhibitory. (44,46)

2) Prostacyclin

Prostacyclin, or 9-deoxy-6,9-epoxy-5-$PGF_1\alpha$, was first discovered in 1976. Its platelet aggregation inhibiting action was the basis for its bioassay even before the chemical structure was elucidated. (50,51) Production of PGI_2 was shown to be highest in endothelial cells, (52,53) and it is the major product of arachidonic acid metabolism in the corpus luteum, human follicular tissues, uterus, kid-

neys, and bovine seminal vescicles.

Prostacyclin has a half life of two to three minutes at 37 C and pH 7.5. It is known to break down nonenzymaticly into its inactive metabolite 6-keto $PGF_1\alpha$. (45,51) One group (43,54) suggested that not only does PGI_2 break down into several metabolites besides 6-keto $PGF_1\alpha$, but that one of its metabolites, PGE_1, may be the true vasodiating and platelet aggregation inhibiting substance (fig. 3.) This is currently a controversial area, and supporting data for this opinion has not been repeated by other labs.

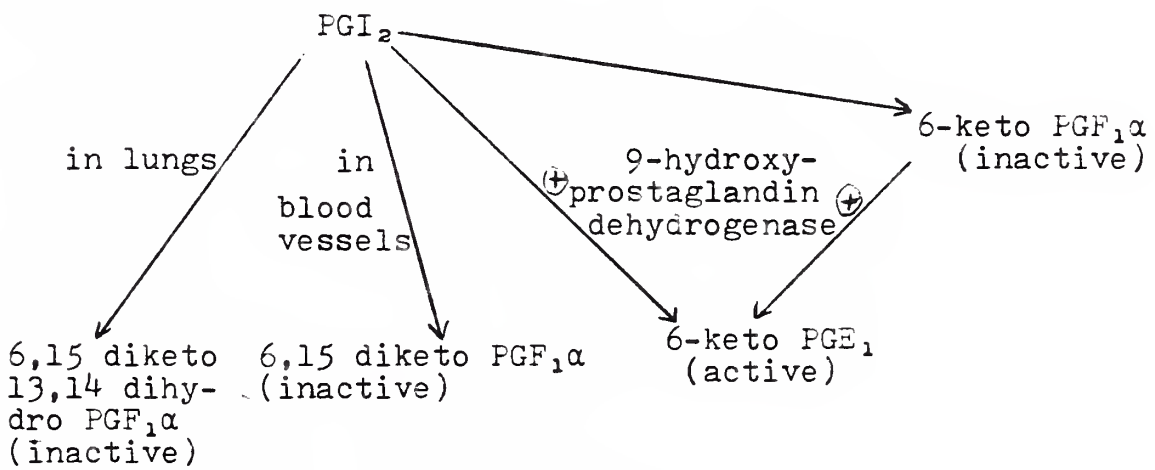


fig. 3. Proposed PGI_2 Metabolism (43)

Although most of the prostaglandins are thought to act as autocoids (locally acting hormones), it is possible that PGI_2 acts as a circulating hormone. The "primary" prostaglandins, such as PGE_2, PGF_2, and PGA, are rapidly converted in the lungs by 15-hydroxyprostaglandin

dehydrogenase. However, PGI_2 has been shown to pass through the lungs without breakdown. Venous PGI_2 infusion, but not PGE_2 infusion, causes systemic hypotension in dogs, indicating recirculation. (20) Prostacyclin may even be released from the lung endothelium.(44)

Besides platelet aggregation inhibition and vasodilation, PGI_2 has been shown to be involved in at least two regulatory mechanisms. The kallikrein-bradykinin system and the renin-aldosterone angiotensin system both have some apparent regulation by prostaglandins, most probably prostacyclin. The kallikrein-bradykinin system's involvement with prostaglandins has not been completely elucidated.

Prostacyclin or prostacyclin-like compounds are known to influence the renin-angiotensin-aldosterone system in at least three ways: 1) Prostacyclin causes release of renin secretion from the kidney and possibly the gravid uterus(43, 56); 2)prostaglandins are released from the kidney by angiotensin II; and 3) Prostaglandins have been implicated in regulating vascular sensitivity to the hypertensive effect of angiotensin II (fig. 4) (43,56,57,58)

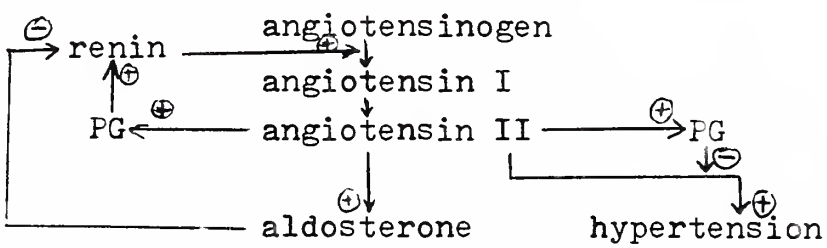


fig. 4. Effects of Prostaglandins on the renin-aldosterone - angiotensin system.

Secretion of renin from the renal cortex has been shown to be stimulated by the direct action of PGI_2. (59) Intrarenal infusion of PGI_2 at 10^{-8} g/kg/ml leads to 180% increase in renin secretion and a 35% increase in renal blood flow. PGE_2 and PGD_2 had lesser pressor effects. (20) Prostacyclin infusion has been shown to increase renin activity in normal men. (56)

Prostacyclin and /or prostacyclin-like substances are thought to regulate angiotensin II output and its hypertensive effects, and so help maintain renal blood flow during variations in blood pressure and total blood volume. PGE and PGI_2 output from the renal medulla increase with infusion of angiotensin II. (19) Prostacyclin-like material has been shown to be released from the kidney with infusion of angiotensin II, leading to blunted vasoconstrictor and antidiuretic effects. When indomethacin is given to block prostaglandin synthesis, the vasoconstrictor and antidiuretic actions are no longer blunted. (6 ,62)

Prostacyclin (and/or another prostaglandin) could then regulate the system as follows: Hypotension and/ or hypovolemia signal renin release from the kidney (via prostacyclin.) This leads to increased angiotensin II and aldosterone levels, with resultant increased water and salt retension, and vasoconstriction. In the kidney, angiotensin II triggers prostaglandin release (mostly PGE and PGI_2), which acts locally as a vasodilator and as stimulus for further renin release, with perhaps slight systemic action. The

systemic vasoconstriction and renal vasodilation would protect the renal blood supply during periods of hemodynamic stress. Differential output of prostaglandins may also effect zonal distribution of renal blood flow. (43)

B. Prostaglandins in Pregnancy and in Preeclampsia

1) Prostaglandin E

Prostaglandins have been proposed as the regulators of uterine blood flow for many years. (63,59,65) Prostaglandins of the E series (PGE_1,PGE_2, etc.) are potent vasodilators. (Because most assays are unable to distinguish the various E prostaglandins, they will be referred to as one group-PGE.) PGE must exert its effects locally, because of rapid metabolism in the lungs by 15-hydroxyprostaglandin dehydrogenase. Up to 90% is metabolized in one pass. These two characteristics (vasoconstriction and rapid breakdown) make PGE an ideal substance for regulating blood flow within an organ.

Autoregulation of renal blood flow by PGE and PGI_2 has been discussed. Like the kidney, the gravid uterus is a major source of PGI_2, PGE, and renin. These compounds could together regulate uterine blood flow by the same mechanism as in the kidney. (57,65) Several studies have shown decreased uterine blood flow in animals given cyclooxygenase

inhibitors. (66,67,68)

There have been many reports of PGE levels in peripheral and uterine venous plasma during normal pregnancy. Results varied in both absolute plasma concentrations and in relative changes noted throughout pregnancy. (69) Amniotic fluid levels of PGE have consistently been shown to increase throughout pregnancy, with highest levels occuring in labor. (69)

If PGE is at least partially responsible for the uterine blood supply, a change in its metabolic rate or activity may cause some of the abnormalities seen in preeclampsia. Some researchers have proposed that a decrease in PGE, with a relative increase in vasoconstricting $PGF_2\alpha$, may have a central role in the disease. Speroff (65) proposed the mechansim shown below (fig. 5):

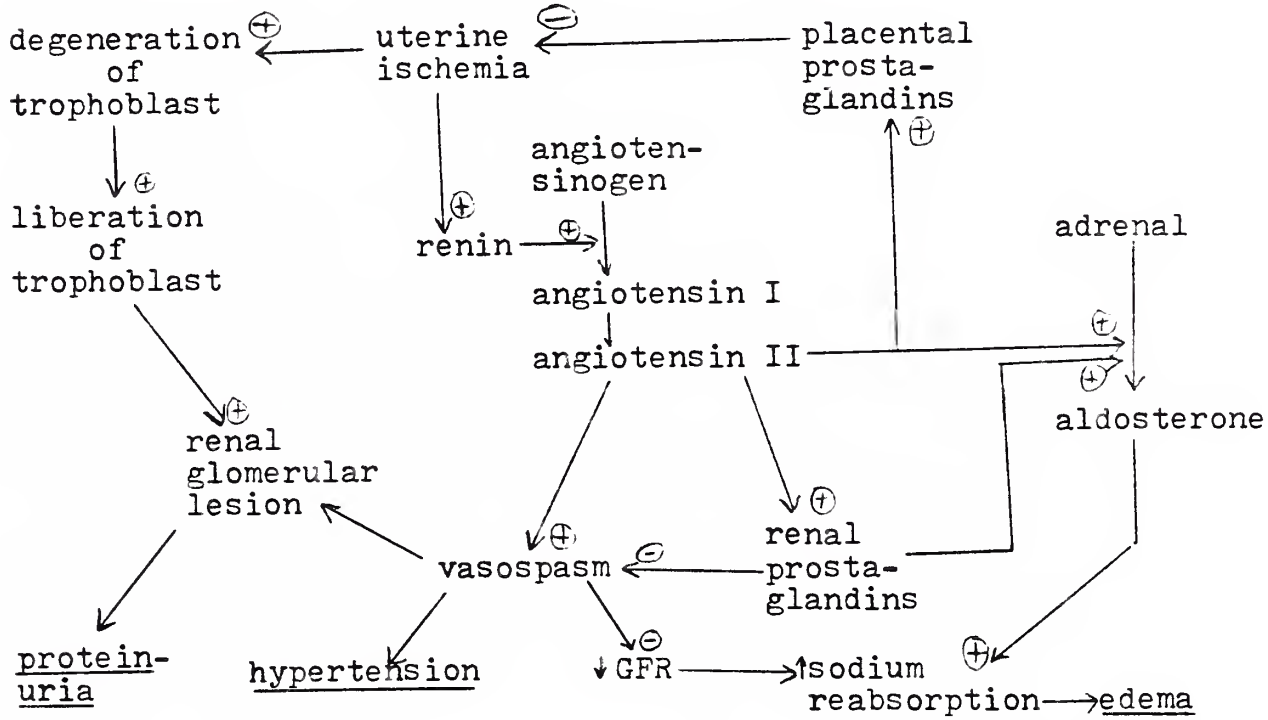


fig. 5. Proposed role of prostaglandins in preeclampsia.

There are several important problems with this mechanism. 1) PGE has been shown not to cause systemic drops in blood pressure even upon intravenous infusion of large amounts. (20) 2) There is a decrease in the renin-aldosterone-angiotensin system found in preeclampsia that is associated with edema, not an increase. 3) The changes in the coagulation system are not included. This mechanism was proposed before the discovery of TXA_2 and PGI_2.

There are few reports of PGE measurement in patients with preeclampsia. Demers and Gabbe (59) measured PGE by radioimmunoassay in the placental tissues of preeclamptics. They found lowered PGE and elevated $PGF_2\alpha$, but these levels did not correlate with the severity of the disease. Robinson et al. found that PGE was significantly decreased in the amnion, chorion,and decidua of women with preeclampsia. (70) It is doubtful that the results of these two studies describe PGE changes due to preeclampsia. They probably do not reflect prostaglandin levels prior to the onset of labor, because prostaglandins are known to be intimately involved in labor.(69,70,71)

Another group measured the ability of the placental vessels to synthesize PGE and PGF. (72) No significant difference was found between normals and preeclamptics.

Although perhaps not central to the disease process, PGE may still play an important role in preventing the uterine ischemia thought to occur in preeclampsia-eclamp-

sia. The true production rate and effects of PGE in pregnancy and in preeclampsia remain unclear.

2) Thromboxanes in Pregnancy and Preeclampsia

Changes in vasoconstriction and platelet aggregation are known to occur in pregnancy and preeclampsia. Since these are the actions of thromboxane A_2, it is possible that they could be due to a change in its synthesis and/or action. In any biological systems in which TXA_2 or its antagonist, PGI_2, are implicated, it would be important to document production or concentrations of both compounds.

An increase in TXA_2 throughout pregnancy was measured by thin layer chromatography, radioimmunoassay, and gas chromatography-mass spectrometry (GC-MS.) (73) Levels were shown to double between late pregnancy (28-36 weeks) and term. TXA_2 (as determined by its inactive metabolite TXB_2) remained at the same high level during the first stage of labor, then rose again during delivery. Mode of delivery did not influence TXB_2 concentrations. Preterm labor had levels consistent with delivery but not with dates. TXB_2 levels in preeclamptic women between 28 and 36 weeks gestation were not different from those of normal women of the same gestational age, and did not correlate with platelet count, factor VIII, or β-thromboglobulin. (70)

3) Prostacyclin in Pregnancy

Prostacyclin may have several actions in the maintainence of the altered physiological state of normal pregnancy, such as maintaining vasodilation, vascular insensitivity to angiotensin II, increased uterine blood flow, and increased levels of renin, angiotensin, and aldosterone.

Dose-dependent systemic vasodilation with resultant decreased blood pressure has been shown with intravenous infusion of PGI_2. (20)

The insensitivity to the hypertensive effects of angiotensin II have been linked to prostaglandins, most likely PGI_2. Dietary deprivation of essential fatty acids in rabbits during late pregnancy led to increased angiotensin II sensitivity. (75) Infusion of angiotensin II in pregnant monkeys or dogs in the third trimester caused increased prostaglandin release and increased uterine blood flow. Simultaneous indomethacin infusion reversed these effects and caused an increased pressor response. (66,68) Pregnant sheep given indomethacin developed significantly increased blood pressure and decreased blood flow to the kidneys, uterus, and the coteledons of the placenta. (67) Vascular sensitivity to angiotensin II was increased in normal pregnant women after taking indomethacin or aspirin. (58) Women taking aspirin regularly during pregnancy were found to have lower birthweight babies and an increased rate of stillborns compared to those who did not take aspir-

in. (76) Thus blockage of prostaglandin synthesis is linked with increased angiotensin II vascular response, decreased uterine blood flow, and uteroplacental ischemia. Although PGI_2 levels were not specifically measured, it is known that PGI_2 is the major product of arachidonic acid metabolism in the endothelium, uterus, and kidneys.(44)

Prostacyclin is known to be involved in the renin-angiotensin-aldosterone system in at least two other ways. 1) Prostacyclin leads to renin release. 2) Angiotensin II causes prostaglandin release (PGI_2 and PGE) from the kidney and probably the pregnant uterus.

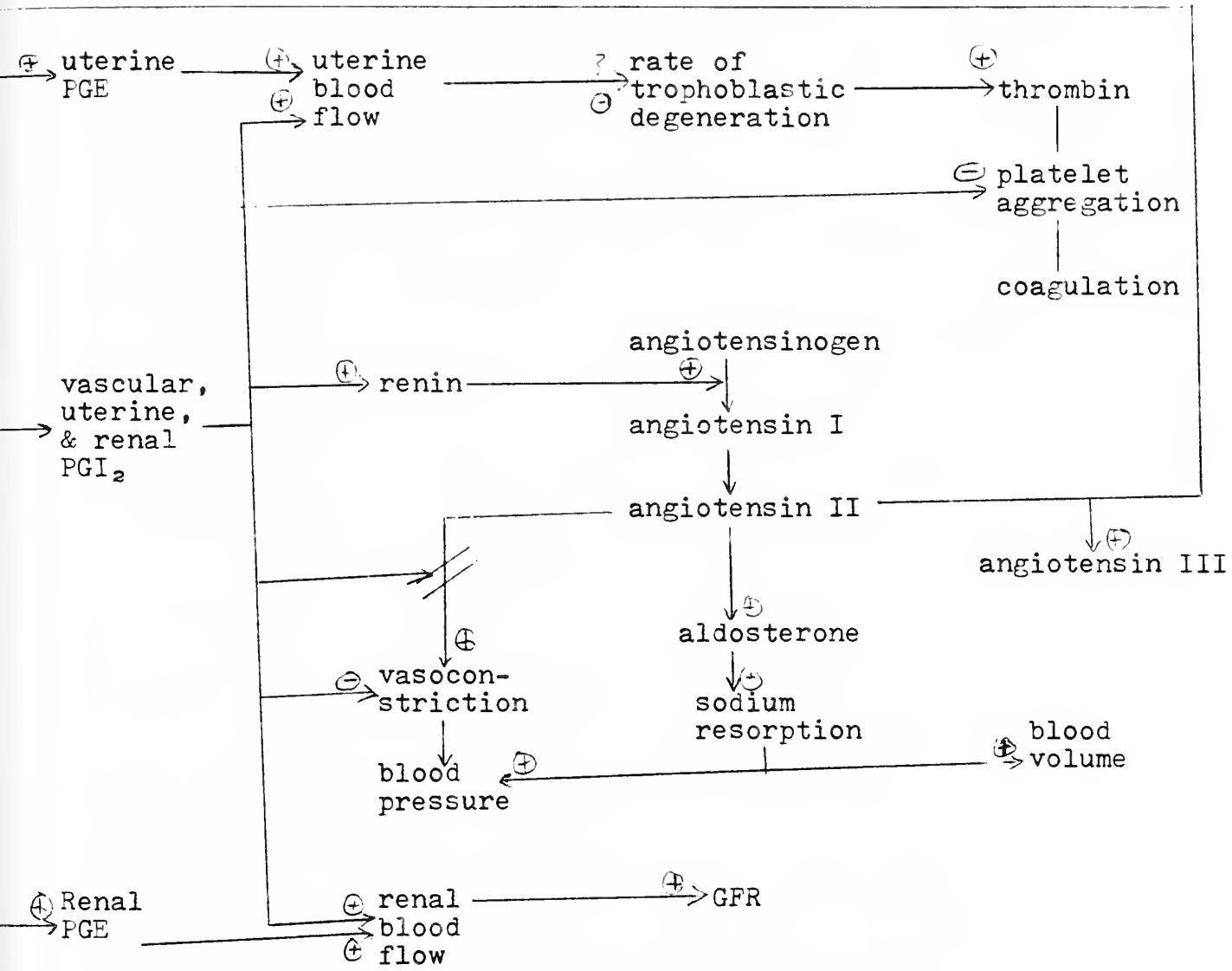
Possible actions of prostacyclin in normal pregnancy might then be summarized as in figure 6.

Several groups have measured prostacyclin, or its metabolite, 6-keto $PGF_1\alpha$, in tissues of animals having normal pregnancies;

Terragno, et al. found 6-keto $PGF_1\alpha$ to be the major metabolite of arachidonic acid metabolism in vascular tissue from pregnant and nonpregnant cows. (77) Highest levels were in fetal vascular tissue.

Hamberg et al. found 6-keto $PGF_1\alpha$ to be the major product of ^{14}C-aracidonic acid in isolated human umbilicical arteries. (78) Human umbilical vessels and placental vessels were shown to have greater PGI_2 activity than vessels from normal adults.(79)

fig. 6. Possible actions of prostacyclin in pregnancy.



Homogenates of human cervix were incubated with ^{14}C-arachidonic acid. The production of 6-keto $PGF_1\alpha$ (the major metabolite) was six times greater per net weight in the ripening cervix than in the nonpregnant cervix. (80)

Plasma levels of 6-keto $PGF_1\alpha$ have been reported to increase during normal pregnancy. Lewis et al. (77) found significantly higher 6-keto $PGF_1\alpha$ in women in late pregnancy (later than 28 weeks) and in the purperium (less than 7 days postpartum) than in those in early pregnancy (before twelve weeks) or nonpregnant women. Bolton et al. (81) measured circulating 6-keto $PGF_1\alpha$ longitudinally in twelve women throughout pregnancy. They found plasma levels to be highest at 18 to 22 weeks gestation, then decreased to early pregnant levels by 34 to 38 weeks. They published no normal value for nonpregnant women.

One report gives contradictory findings. Ylikorkala et al. (82) found no difference between pregnent and nonpregnant values of plasma 6-keto $PGF_1\alpha$, and did not find any changes with gestational age or other clinical parameters. All three reports of peripheral 6-keto $PGF_1\alpha$ used radioimmunoassay for detection. It appears that the true changes in peripheral PGI_2 in normal pregnancy, if any, remain unknown.

4) Prostacyclin in Preeclampsia-eclampsia

A decrease in circulating prostacyclin has been suggested as the major pathway for the development of preeclampsia by several investigators. (83,84,85) A decrease in PGI_2 during pregnancy, either as the result of vascular changes in the placenta, or as a primary event, could lead to the following pathological changes:

1) increased angiotensin II sensitivity

2) decreased renin-angiotensin-aldosterone levels due to lower PGI_2 stimulation of renin production.

3) Decreased blood volume and edema formation due to hypertension and decreased aldosterone, which lowers sodium resorption and causes saltand water movement into the extravascular space.

4) higher blood pressure due to both increased angiotensin II hypertensive effects, and an inbalance in the TXA_2/ PGI_2 ratio favoring vasoconstriction.

5) decreased renal blood flow and glomerular filtration rate due to local and systemic vasoconstriction and lowered intravascular volume.

These changes could be represented as in figure 7.

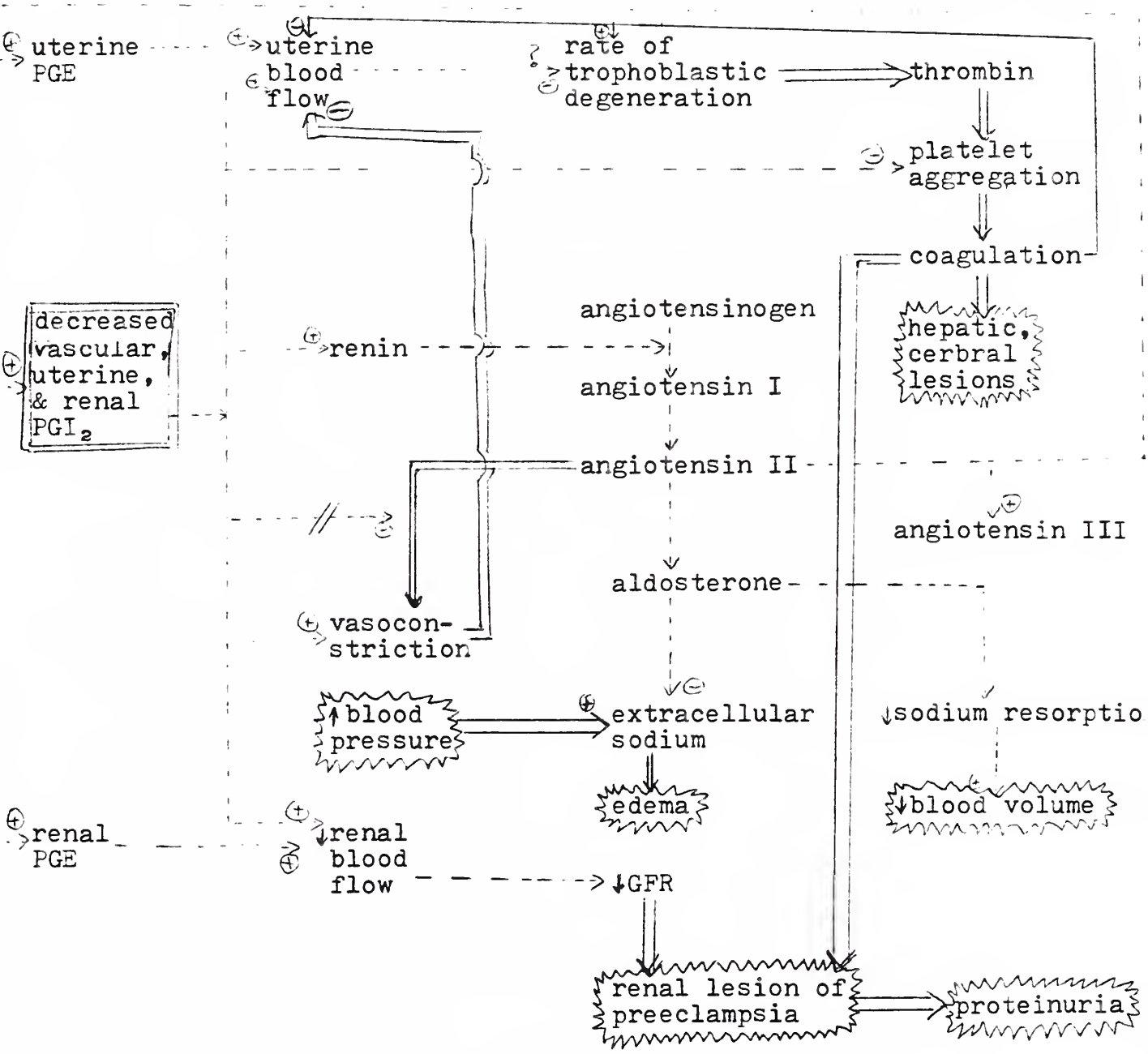
This concept is similar to the "inner vicious circle" model desribed by Page.(86) In the presence of predisposing factors (genetic susceptibility,smaller uterine vessels of the primigravida, multiple pregnancy, hydatidiform mole, etc.), pregnancy overstresses the ability of the uterine

fig. 7. Proposed effect of decreased prostacyclin in preeclampsia.

⟹ - increased action

- - → - decreased action

∿∿∿ - increase in a state or appearance of clinical sign



vasculature to supply blood to the fetoplacental unit, the uterine blood supply being regulated by PGI_2. Insufficient blood supply to the placenta causes increased trophoblastic tissue and thrombin release, leading to increased intravascular coagulation, less uterine blood flow, and eventually the other concomitants of preeclampsia. Once this positive feedback loop ("inner circle") is started, it is difficult to stop. The process currently can only be stopped clinically by removal of the uteroplacental unit. If the decreased prostacyclin theory is correct, perhaps the vicious circle may be broken by administration of prostacyclin, rather than delivery of a pre-term infant.

It is also possible that PGI_2 deficiency could be important only in the most severe cases of preeclampsia - those fifteen percent with thrombocytopenia. At Yale-New Haven Hospital in 1980, most of the maternal and perinatal morbidity and mortality were in women with thrombocytopenia. Increased hypercoaguability from a TXA_2/PGI_2 inbalance could be the operative mechanism responsible for thrombi formation in diffuse organs. Excessive platelet consumption may render a patient so thrombocytopenic that hemostatic mechanisms are compromised. In the hypertensive patient, this may predispose her to the development of intracranial hemorrhage, one of the major causes of death in preeclampsia-eclampsia.

Several lines of evidence suggest that a deficiency of prostacyclin may exist in preeclampsia:

The rate of PGI_2 production in umbilical arteries and placental veins at delivery has been shown to be significantly lower in preeclamptics than in controls.(83,84,85,87, 88) A decrease was also found in other chronic uterine ischemic states.(intrauterine growth retardation and essential hypertension.) Measurement was made with bioassay, radioimmunoassay, and /or gas chromatography-mass spectrometry.

Amniotic fluid in five preeclamptics had significantly lower PGI_2 than in 15 women with uncomplicated pregnancies. (89) Another study found lower amniotic PGI_2 in severe preeclampsia (N-13) as compared to normal pregnancy (N-27), but not in mild preeclampsia, diabetes, rhesus-immunization, or uncomplicated intrauterine growth retardation (IUGR.) (90) Amniotic TXB_2 levels were not different among any of the groups.

These results indicate a decrease occurs in uteroplacental prostacyclin production. To implicate PGI_2 as responsible for many of the systemic manifestations of preeclampsia, a decrease in peripheral levels must be shown. Two case reports have shown PGI_2 levels to drop in preeclampsia. One longitudinal study showed a precipitous drop in plasma prostacyclin five weeks before the onset of clinical preeclampsia in a woman who had had recurrent fetal loss from

this disease. (91) PGI_2 infusion was begun in the woman when the clinical symptoms of preeclampsia developed. The blood pressure dropped slighty, and the woman's pulse rose fifteen beats per minute in response to the drug. Fetal death occured after four days of treatment. It was thought by the authors that a PGI_2 infusion beginning at the time of the drop in plasma prostacyclin might have lead to a more favorable outcome.

The same group treated a 29 year old woman with infusion of PGI_2 for recurrent preeclampsia, after blood pressure was unable to be controlled by conventional antihypertensive medicines. Although plasma 6-keto $PGF_1\alpha$ levels were apparenty not measured, the patient responded to the drug with a dramatic decrease in diastolic pressure (from 140 mm Hg to 80 mm Hg.) Three episodes of fetal bradicardia lasting one to two minutes each occured on the fourth day of PGI_2 administration, so the fetus was delivered by cesearian section at $27\frac{1}{2}$ weeks. When the infusion was momentarily stopped for indution of anesthesia, the patient's blood pressure rose to 200/110 mm Hg. After the infusion was restarted, the blood pressure returned to 130/80. The infant has since done well. (92)

Remuzzi et al. (93) noted a decrease in a poorly defined "prostacyclin stimulating factor" in late normal pregnancy, but saw an increase to the nonpregnant values in preeclampsia.

The only published study of maternal plasma 6-keto $PGF_1\alpha$ in preeclampsia (94) found no differences in these levels in preeclamptics women (266.4 pg/ml) versus normals (267.0 pg/nl), or those with other complications of pregnancy (diabetes, twins, premature labor, bleeding, IUGR). However, this study is from the same group that found no difference in plasma 6-keto $PGF_1\alpha$ levels in preganat women versus nonpregnant women. (82) There are several problems with the study itself. Their measurement technique (radio-immunoassay) was never verified by gas chromatography-mass spectrometry, or any other methods. Thus the method may be so nonspecific that changes in actual 6-keto $PGF_1\alpha$ may be masked. A biological blank was never described (see next section). Participant selection was questionable. "Pre-eclamptics" were defined as those patients with blood pressures over 140/90 mm Hg. No consideration was given to proteinuria, edema, or coagulation changes. Whether patients with essential hypertension or superimposed pre-eclampsia are included in the group is not specified. These flaws,and the fact that only one study measuring 6-keto $PGF_1\alpha$ in preeclamptics has been reported to date, make it important to repeat the study.

III. Purpose of Study

The possibility that there is decreased prostacyclin (PGI_2) production and action in preeclampsia-eclampsia is currently being examined by several laboratories. Beacuse of prostacyclin's various actions (vasodilation, platelet aggregation inhibition, and possible role in the renin-angiotensin-aldosterone system), an increase in the peripheral activity of this chemical could explain much of the altered physiology of normal pregnancy. A relative decrease in peripheral and local activity could theoretically tie together the various pathophysiological findings of preeclampsia.

Therefore, 6-keto $PGF_1\alpha$, the stable nonenzymatic metabolite of prostacyclin, was measured in the plasma of women with and without preeclampsia, to determine if there were any changes in peripheral concentrations of the chemical in these conditions.

IV. Techniques for Prostacyclin Measurement

There are four major techniques for prostacyclin measurement (and for all prostaglandins)(42,43):

1) Gas Chromatograhy-Mass Spectrometry (GC-MS) Sensitive, specific, and definitive, but also time consuming and requiring expensive, delicate equipment that is not available to all investigators. It is the standard to which other methods are compared.

2) Radioimmunoassay (RIA) 6-keto $PGF_1\alpha$, the stable non-enzymatic metabolite of prostacyclin, is detected. The method is extremely sensitive (to the picogram range) but is hampered by a lack of specificity. Relatively inexpensive and simple.

3) Bioassay Less sensitive and less specific, it was the original detection method for both TXA_2 and PGI_2 before their chemical structures were known. This is the only method that detects prostacyclin directly, instead of its metabolite. Two different types of assay are used:

superfusion bioassay - Blood is withdrawn from an animal by a pump, run over three isolated assay organs, and returned to the animal. Smooth muscle relaxation is correlated with prostacyclin concentration.

platelet aggregation inhibition bioassay A biological sample such as citrated plasma is added to platelet-rich

plasma. The sample's ability to inhibit ADP-induced platelet aggregation is measured spectrophotometricly. (95) Calibrated by CG-MS, inhibition can be used to measure prostacyclin activity. Assurance that PGI_2 is the active substance is made by demonstating its stability at 0° C and basic pH, but not at physiologic conditions.

4) ^{14}C-Arachidonic acid Incorporation - Chromatography

Used to measure the ability of a tissue to produce prostacyclin. ^{14}C-aracidonic acid is added to a washed tissue sample and incubated. The radioactive products are separated by chromatographic isolation and the relative amounts of each product are measured with a scintillation counter

Radioimmunoassay and bioassay are the common and most practical methods for prostacyclin measurement. Bioassay has been used for a longer time, but has the disadvantages of lack of sensitivty, lack of specifity, and the need to run each sample immediately after it is obtained. RIA was chosen for experimental PGI_2 determinations in this study beause of its availability, relative simplicity, low cost, and sensitivity.

A. Radioimmunoassay

The basis of RIA is competitive binding of labeled and unlabeled antigen to a specific antibody. Unlabeled antigen (the sample) is mixed with labeled (radioactive) antigan. The two are allowed to react with a limiting and con-

stant amount of specific antibody. As the amount of unlabeled antigen is increased, less binding of the labeled antigen occurs. Bound(antigen-antibody complexes)antigen is commonly separated from the free antigen by adsorption of free antigen onto activated charcoal. The charcoal is then removed by centrifugation.

The radioactivity of the antigen-antibody complexes is measured. A standard (dose-response) curve is made by assaying known amounts of the substance. The amount of antigen in each unknown sample is interpolated from the dose-response curve. (96)

For the particular assay used in this experiment, the antigen was 6-keto $PGF_1\alpha$. The labeled antigen was ^3H-6-keto $PGF_1\alpha$. Radioactivity was measured by adding phosfluorescence coctail that releases photons when hit by β particles from the radioactive antigen.

The major problem with RIA is lack of specificity. This is caused by nonspecific binding of other substances by the antibody. In prostaglandin assays, this could be due to other prostaglandins, related chemicals (such as fatty acids) or to unrelated compounds. The techniques of sample collection and purification can greatly affect the amount of materials that may bind nonspecificly to the antibody, and so are critical to the assay.

2) Sample collection

All researchers using the 6-keto $PGF_1\alpha$ RIA in plasma agree that blood samples must be collected in cold tubes or syringes containg anticoagulant and a cyclooxygenase inhibitor to stop further arachidonic acid metabolism. Some workers use heparin (97,98,99) but others use EDTA (ethylenediaminotatraactetate) as an anticoagulant. (81,100) Heparin has been reported to cause falsely elevated values for the 6-keto $PGF_1\alpha$ measurement due to inhibition of antigen-antibody binding. (96,100) Indomethacin is the most commonly used cyclooxygenase inhibitor, but aspirin, although less potent, has been shown to have comparable results.

Samples are then quickly centrifuged. The plasma is removed and stored at -20°C. No data is available on the length of time that a sample may be stored without changing its binding characteristics, but most papers specify that samples were assayed within a month of collection.

3) Sample Purification

Considerable controversy exists over the need to purify a plasma sample prior to RIA.

When RIA of a new arachidonic acid metabolite is being developed, purification by several steps has been traditionally been used to remove any other materials that might bind to the antibody. Purification by extrac-

tion to remove more polar chemicals, followed by silica gel column chromatography and sometimes high pressure liquid chromatography (HPLC), has been used. (101,102)

HPLC has been shown to be unnecessary for use with the RIA of several prostanoids. (102) and was not used in any of the reports descibing 6-keto $PGF_1\alpha$ in plasma.

Silica gel column chromatography theoretically would improve specificity by removing most of the other prostaglandins. However, preliminary studies done during this project showed that not only was 30 - 40% of the 6-keto $PGF_1\alpha$ lost during this step, but the solvents used in the procedure (especially benzene) contained material which cross-reacted with the antibody, causing falsely elevated measurements. This result was confirmed by Hall. (103)

Most often, extraction is the only purification procedure considered necessary. Extraction is done to dissociate prostacyclin from albumin, remove polar substances, and to concentrate the sample so that lower concentrations of the compound may be detected. But several groups have found that extraction adds more impurities to the sample. (96,104) Morris, Sherman, and Sheppardson (100) noted that when samples derived from comparable volumes of reagent blanks were assayed with diethyl ether, ethyl acetate, or chloroform, RIA measurements were greater than those of unextracted plasma. Mitchell (106) found that ethyl acetate extraction of acidified samples caused the formation of

"3 or 4 other compounds"- most of which reacted to the antibody. One group, which used ethyl acetate extraction, reported water and buffer blanks of 7.8pg/ml by assay. However, it is unclear whether these blanks were subjected to the extraction procedure. (97)

Because of these difficulties with plasma purification techniques, several groups suggest that plasma may be directly assayed. (96,100,104) This approach would require the development of a "biological blank."

Samples must be in the same milleu as the standards to which they are compared. Extracted samples are reconstituted in buffer. Controls and standards are in buffer. If samples are not extracted, the controls and standards must be in the same biological matrix (plasma), but this matrix must not contain any 6-keto $PGF_1\alpha$ or cross-reacting materials. Attempts have been made to prepare this "biological blank" without success. (96) Morris et al. (100) have used the plasma from subjects treated with 1800mg of aspirin a day for 3 days. No mention of the plasma blank's actual measurement by RIA was made. Hall described charcoal stipped plasma blanks reading 40-50 pg/ml. (103) Mitchell tried removing cross-reacting substances several ways: feeding plasma donors aspirin for several days before sampling, treating plasma with charcoal before use, dializing the plasma, and precipitating out the 6-keto $PGF_1\alpha$ with a specific antiserum. All of these techniques changed the bind-

ing characteristics of the plasma used for the biological blank, and lead to negative values for plasma samples compared with standards made up in the treated charcoal.

It appears that all of the methods of sample preparation have serious drawbacks that have not been overcome.

4) Assay Verification

It is important to validate the results of radioimmunoasay by several parameters.

1)Specificity This is determined by the cross-reacting compounds and by nonspecific binding to the antibody. The latter is difficult to determine, but may be approached by measuring blanks of different solvents used in the assay. Cross-reactivity is calculated as:

$$\frac{\text{amount of specific antigen for 50\% displacement}}{\text{cross-reacting substance needed for displacement}}$$

2) Sensitivity Three definitions are commonly used- i) the amount of antigen needed for a certain percentage of displacement of the labeled antigen (50% displacement, or mass for logit=0, is commonly quoted), ii) slope of the dose response curve, and iii) "least detectable mass"- mass resulting in a response two standard deviations from the blank's value.

3) Precision How closely repeated measurements of the same sample agree. The coefficient of variation is often used as an index of precision.

$$\text{coeffitient of variation} = \frac{\text{standard devation}}{\text{mean of samples}} \times 100\%$$

4) Accuracy How close the measured values are to the actual value. This can be assessed in two ways - i) measurement of the samples by an independent method, such as GC-MS. ii) Assaying known concentrations of standard and comparing the assay result with the known amount.

5) Parallelism Each sample must, on dilution, yield a curve that is parallel to the standard curve. This test can be done with the test for accuracy. A known amount of antigen can be serially diluted, and the slope of the line of measured vs. known amounts calculated.

Without these verifications, the data cannot be considered reliable. Even if all the tests done within an assay are acceptable, the data could still be misleading. For example, a substance may have low cross-rectivity, but if it is at very high concentrations compared to the antigen, it would have a significant effect on the assay. Thus it is important each time an assay is developed for a biological system such as plasma, that confirmation be made by another

method, preferably gas chromatography-mass spectrometry.

V. Materials and Methods

Plasma 6-keto $PGF_1\alpha$ was measured by radioimmunoassay in women with preeclampsia-eclampsia, and in women having uncomplicated pregnancies (controls). Two studies were done. Study I had samples from 14 preeclamptic women and 46 controls. All samples were extracted with ethyl acetate. In study II, samples from five preeclamptics and 24 controls were prepared by different methods- ethyl acetate exraction, solid phase extraction, and no extraction.

A. Materials

Indomethacin - Merk, Sharp, and Dohme Research Laboratory, Rahway, New Jersey
Ethanol - 200 proof, Pharmco. Publicker Industries Co. Linfield, Pensylvania
ethyl acetate - spectrophotometric grade, Mallinckrodt, Paris, Kentucky 40361
methanol - spectrophotometric grade, Mallinckrodt, Paris Kentucky 40361
acetic acid - Fischer Scientific, Fair Lawn, New Jersey 07410
trimethylpentane - Spectrophotometric grade, Mallinckrodt, Paris, Kentucky 40361
Phosphomolybdic acid - 3.5% in isopropanol - Brinkmann Inst. Westbiy, N.Y. 11590
Hydrochloric Acid - J.T.Baker Chemical Company Phillipsburg,New Jersey 08865
Sodium phosphate monobasic - Mallinckrodt, St. Louis, Missouri 63164
Sodium Chloride - Fischer Scientific Fair Lawn, New Jersey 07410

Sodium Phosphate Dibasic Heptahydrate - Mallinckrodt,
St. Louis, Missouri 63147
Sodium Azide - Mallinckrodt,
St. Louis, Missouri 63147
Gelatin - Matheson,Coleman, and Bell
Norwood, Ohio 45212
Picofluor scintillation solution for aqueous samples,
Packard Instrument Co. Downers Grove, Illinois 60515
Quantagram thin layer chromatography plates, 5x20 cm
Quantum Industries Fairfield, New Jersey 07006
Isocap Liqiud Scintillation Ssytem , Chicago Nuclear
Chicago, Illinois
Hewlet Packard 9866 minicomputer,
Loveland, Colorado 80537
Heparinized vacutainer tubes,10 ml - Becton-Dickinson,
Rutherford, New Jersey 07070
Charcoal, Norit A - Fischer Scientific Company,
Fair Lwan, New Jersey 07410
Dextran T70, Pharmacia,
Uppsala, Sweden
Water soluble silicone, Siliclad - Clay Adams
Pasippany, Pennsylvania 07054
Borosilicate glass test tubes, 12x75 - Scientific Products
Fair Lawn, New Jersey 07410
^{3}H-6-keto $PGF_1\alpha$, in acetonitrile/water 9:1, New England
Nuclear, Boston, Massachusetts 02118
^{3}H-TXB_2 (5,6,8,9,11,12,14,15 ^{3}H-TXB_2, in ethanol, New
England Nuclear, Boston, Massacusetts 02118
TXB_2 and 6-keto $PGF_1\alpha$ antisera were provided by
Dr. Harold R. Behrman, Professor,Obstetrics and Gynecology and Pharmacolgy, Yale University School of Medicine New Haven Connecticut 06520

B. Methods

Participant Selection All women who were admitted to the Yale-New Haven Hospital labor and maternity floors with the diagnosis of preeclampsia-eclampsia or hypertensive disease of pregnancy were eligible for the study. Participants were selected between November 4,1981 and February 20,1982. Pertinent clinical data was recorded to verify the diagnosis of peeclampsia-eclampsia or superimposed preeclampsia.

Control blood samples were obtained from pregnant women of various gestational ages at the YNHH Women's Center. In both preeclamptics and controls, the participants' permission was obtained according to the Human Investigation Committe Protocol.

Sample Collection ten ml blood samples were taken by venipuncture using a tourniquet into ice-cold heparinized vacutainer tubes containing one microgram of indomethacin. The contents of each tube was gently mixed, immediately placed on ice, and transported to the laboratory (approx. 3 minutes.) Tubes were then centrifuged at 2000xg for 10 minutes at 4°C. Two 2 ml aliquots of plasma were then pipetted off, care being taken not to disturb the buffy coat. Approximately 2000 cpm of purified ^{3}H-6-keto $PGF_1\alpha$ and ^{3}H-TXB_2 in 0.2 ml of Buffer L (described below) were added to each sample for recovery determination. Then each sample was vortexed and stored at -20 C until assay.

Purification of Tritiated Tracers For each tracer, 100μl of trace in ethanol or acetonitrile/water, and 100 μl of nonradiocative standard were then each evaporated under N_2, and resuspended in 50μl of 2:1 chloroform:ethanol. Standard and tracer were spotted on separate columns of the same silica gel thin layer (TLC) plate, and chromatographed in a mixture of 22:4:10 ethyl acetate:acetic acid:

trimethylpentane. The TLC columns were separated, and the column with the standard was developed by spraying with 3.5% phosphomolybdic acid and heating until the standard became visible. The corresponding segment of the column containing tracer was collected into a fiber-glass-stopped pipette, and the tracer was eluted with ethanol.

Preparation of Solvents and Buffers

Buffer L ($Na_2HPO_4 \cdot H_2O$, 0.538 g/l; $Na_2HPO_4 \cdot 7H_2O$, 1.635 g/l; NaN_3, 1g/l; NaCl, 9g/l; and gelatin, 1g/l) was used as the diluent for the recovery tracers.

Charcoal with fines removed was prepared by vigorously mixing 20 g Norit A charcoal with 200 ml distilled water in a graduated cyllinder, then allowing the mixture to settle. The fines were removed from the top of the water by adsorption onto paper towels. The water was decanted, and the charcoal dried overnight.

Dextran Coated Charcoal was prepared by suspending 0.5 g clarcoal with fines removed and 50 micrograms Dextran T-70 in 200 ml buffer L.

Preparation of plasma controls Charcoal-treatin plasma should remove all prostaglandins and so allow a standard curve and biological blank to be made from it, but it is possible that this step might alter the binding characteristics of the plasma. Therefore, plasma standards were made up in both

charcoaled and uncharcoaled plasma. For charcoaled plasma, 250 ml of human plasma was obtained from the Blood Bank. Charcoal with fines removed was added to make a 5% w:v solution. The mixture was mixed at 37 C for two hours, then centrifuged at 2200 rpm for fifteen minutes. The supernatant was filtered through a buchner funnel with filter paper to remove any remaining charcoal.

Using both charcoaled and uncharcoaled plasma, two sets of control plasma standards were made. In each, 6-keto $PGF_1\alpha$ was added to an aliquot and serial dilutions made for final concentrations of 2000,1000,500,250,125, 62.5,and 0 pg/ml of added antigen.

Sample Preparation

Two studies were done. In the first, all samples were prepared by liquid/liquid phase (ethyl acetate) extraction. The assay was run in borosilicate glass test tubes. In Study II, samples from each patient and all standards were prepared by three methods - solid phase extraction, liquid/liquid phase extraction (with ethyl acetate) and using no extraction. Siliconized test tubes were used.

Liquid/liquid Phase Extraction 2 ml plasma samples, with recovery added, were thawed and acidified to pH 3.5 with 0.1 ml of 1.0 N hydrochloric acid. Eight ml of redistilled ethyl acetate were added, and each sample was shaken by hand for five minutes. The phases were separated

by centrifugation at 2200 g and 4 C for 10 minutes. The upper phase was pipetted off and dried under N_2 in a small scintillation vial at room temperature. The sample residues were resuspended in 2.0 ml ethanol and stored at -70 C until assayed.

Solid Phase Extraction C_{18} silica cartriges were attached to disposable syringes, and activiated by passing 2×1 ml of methanol, then 3×1 ml of distilled water through the cartriges. Residual fluid was removed by forcing air through with the syringe between each addition step. The 2 ml plasma sample with recovery trace was added to the cartrige and allowed to go through. Residual sample was removed with air. The column was washed with 2×3 ml of distilled water, and the prostaglandin-containing fraction was eluted with 2×0.5 ml of methanol.

Siliconized test tubes were used for the second study. Borosilicate glass test tubes were immersed for 5 seconds in a 1:100 solution of water soluble silicone, then thoroughly rinsed first in tap water, then distilled water, and allowed to dry.

6-keto PGF,α Radioimmunoassay

The assay was performed according to the New England Nuclear RIA kit instructions, except that unsiliconized tubes were used for study I. Treatment of extracted and unextracted samples were slightly different.

Procedure for extracted samples

Standards of 1000,500,250,100,50,25,and 10 pg/0.1 ml of kit buffer were freshly diluted just prior to assay. Then 0.1 ml of each standard was aliquoted in triplicate into assay tubes. One ml of each extracted unknown sample and plasma standard was aliquoted into an assay tube and dried down under air at room temperature. 0.2 ml (or ten percent) of each sample was stored in a scintillation vial for later estimation of recovery. Total count (s), zero added mass (0) and blank (0ab) tubes were prepared. Reagents were added to the tubes as follows:

	buffer	tracer	antibody
s	900µl	100µl	- µl
0Ab	400	100	-
0	300	100	100
Standards	200	100	100
Plasma controls	300	100	100
Samples	300	100	100

Thus all tubes except s had a final total volume of 500 microliters. The tubes were vortexed for 5 seconds each and allowed to incubate for 16 hours at 4 C.

After incubation, all tubes were placed in an ice

bath for ten minutes. 500 microliters of iced charcoal suspension were added to each tube except s within one minute. All tubes remained in the ice bath for an additional fifteen minutes, and then was centrifuged at 1000 g at 4 C for 10 minutes. The supernatant fraction was decanted into scintillation vials using consistent technique. 3.5 ml of Pico-Fluor scintillation cocktail was added, and the mixture was vortexed for 2-4 seconds. The samples were then put in the scintillation counter for radioactivity measurement.

Procedure for Unextracted Samples

Because the unextracted samples were in plasma rather than buffer, standards could not be in buffer. Therefore, all standrds were freshly made up in charcoaled plasma. 0.6 ml of each unknown sample and each plasma control were aliquoted into an assay tube. s, OAb, and O tubes were prepared. Reagents were added to the tubes as follows:

	buffer	tracer	antibody
s	1200 µl	100 µl	- µl
OAb	700	100	-
O	600	100	100
Standards	500	100	100
Plasma Controls	-	100	100
Samples	-	100	100

Thus all tubes excet s had a final volume of 800 microliers. The rest of the procedure was unchanged.

C. Calculation of Results

All calculations were made using the Hewlett Packard 9380 minicomputer and a previously prepared program. (107) The standard curve was obtained from the standards in buffer using weighted linear regression analysis and the logit/ln transformation of Rodbard. (108)

A scatchard plot of bound/free vs. bound, or logit-log$\left(\frac{b/b_o}{(1-b/b_o}\right)$ vs. ln (dose of standard in pg) was automatically printed. The weighted linear regression line was calculated between the lowest linear dose and the dose just producing over logit-0 displacement (or 50% displacement of th tracer). The slope of this line affinity.

The transformation yields a straight line defined by the equation:

$$\ln(\text{dose}) = (\text{affinity}) * \log\left(\frac{b/b_o}{1-b/b_o}\right) - \ln(\text{mass for logit}=0)$$

% Recovery was calculated from C (the cpm of the 10% aliquot of the sample *10) as:

$$\%\ \text{recovery} = \frac{C}{\text{total cpm added for recovery determination}}$$

Calculation of Unknowns was determined from the standard curve transformation by the computer program, which automatically corrected for the mass and added counts of the recovery tracer. Total corrected count, corrected B_o, and

corrected logit were calculated as follows:

corrected total - s - C F

corrected B_o - ((total count)/s)*B_o

corrected OAb (blank)- ((OAb-background)*corrected total - background

B - cpm in assay tube - corrected OAb

$$\text{corrected logit} - \ln \frac{(B/\text{corrected } B_o)}{(1-b/\text{corrected } B_o)}$$

where s - counts per minute (cpm) of the s tubes

C - cpm in recovery vail * 10

F - decimal fraction of the sample aliquoted into the assay tube

B_o - cpm in the zero added mass (0) tube, corrected for Oab

OAb - cpm in the zero antibody (OAb) tubes

All values are in counts per minute (cpm) and corrected for background (cpm measured when empty vail is counted).

From the corrected logit, the total mass was determined by interpolation of the logit/ln transformation. Correction for systemic error due to mass added with recovery tracer was computed as follows:

$$\text{corrected mass} - \text{measured mass} - \frac{\text{total cpm of tracer}}{\text{specific activity} * CE} \quad 100\%$$

where CE - counting efficiency of the scintillation counter, The specific activity of the tracer is in dpm/pg.

Statistical Analysis The statistical significance of the difference in plasma 6-keto $PGF_1\alpha$ between two clinically defined groups was found by the Student's t test:

$$S_{d_{\bar{x}}} = \sqrt{\frac{\Sigma(x_2^2) - \Sigma(x_1^2) - N\bar{x}_2 - N\bar{x}_1}{(N_1 + N_2 - 2)}} \left(\frac{1}{N_1} - \frac{1}{N_2} \right)$$

$$t = \frac{\bar{x}_1 - \bar{x}_2}{S_{d_{\bar{x}}}}$$

where S_{d_x} is the standard error of the difference of the means, N_1 = the number of data points for group 1, N_2 = the number of data points for group 2, and x_1 and x_2 are the values of 6-keto $PGF_1\alpha$ for gropus 1 and 2, respectively.

For testing ths significance of the pearson product-moment correlation between two variables x and y, the following equations were used:

$$r = \frac{m\sigma x}{\sigma y} = \frac{N\Sigma XY - N\Sigma X\Sigma Y}{(N\Sigma X^2 - (\Sigma X)^2)^{1/2} \left(N\Sigma Y^2 - (\Sigma Y)^2\right)^{1/2}}$$

$$t = \frac{r\sqrt{N-2}}{(1 - r^2)^{1/2}}$$

where r = correlation coefficient,
N = the number of paired data points
m = the slope of the correlation line
σ_x = the standard deviation of the x values
σ_y = the standard deviation of the y values

The statistical significance of t for degrees of freedom(N-2) was found in a standard statistics text. (109)

VI. RESULTS

A. Assay Parameters for Study I.

Study I. All samples prepared by ethyl acetate extraction.

$\%B_0$ (Binding with no unlabeled antigen) - 42.2%

Correlation of logit vs. ln dose r^2 - 0.985

Specificity Cross-reactivity of some related compounds (data from New England Nuclear Corporation):

6-keto $PGF_1\alpha$	100%
$PGF_2\alpha$	2.7%
PGE_2	2.0%
TXB_2	0.1%
PGA_2	0.1%
PGA_1	0.3%

Nonspecific binding of assay solvents and blanks:

Substance	"6-keto $PGF_1\alpha$"
ethanol 2 ml	12.0 pg/ml
ethyl acetate 8 ml	10.4
HCl 0.1 ml	1.6
distilled water	0-2.1
distilled water (extracted)	84-97.8

Sensitivity - Study I.

1)Mass for logit-0 (mass needed to displace 50% of the labeled antibody - 199.6 pg

2)"Least detectable mass" (mass resulting in a response 2 standard deviations away from the response to a zero dose) - 110.4 pg

Precision and Accuracy

Serial dilution of standards (extracted):

Amount of 6-keto $PGF_1\alpha$ added to solvent	pooled plasma as solvent	distilled water as solvent
0 pg/ml	65 pg/ml	97.8 pg/ml
0	73	84
0	62	-
62.5	93	-
125	74	176
250	-	276
500	70	457
1000	93	529
2000	151	1398
	r^2 - 0.814	r^2 - 0.963
	y-intercept - 68	y-intercept - 89

Kit Controls (specially treated plasma with either 0 or 1000 pg/ml):

	extracted	unextracted
0 pg/ml	284 pg/ml	0
1000 pg/ml	-	1223

Study I.

Serial dilutions of standards (unextracted):

Amount of 6-keto $PGF_1\alpha$ added to pooled plasma	measured 6-keto $PGF_1\alpha$ in pooled plasma solution
0 pg/ml	58 pg/ml
0	74
0	66
62.5	105
125	139
250	198
500	286
1000	663

r^2 - 0.9623

y-intercept - 89

B. Plasma 6-keto $PGF_1\alpha$ Values - Study I.

patient #	age	gestational age	platelet count	plasma 6-keto $PGF_1\alpha$
Preeclamptics-eclamptics(* - severe):				
**001	30	42 weeks	80,000	137 pg/ml
002	20	40	190,000	67
**003	21	32	75,000	72
*004	25	32	170,000	160
*005	31	40	250,000	154
**006	26	28	370,000	220
*007	28	38	210,000	134
**008	21	35	130,000	-
009	32	40	220,000	-
*010	30	42	150,000	160
011	27	41	290,000	197
***012	26	39	290,000	274
**013	34	34	130,000	89
014	19	38	-	126
				mean - 149
				S.D. - 61
Controls(normal patients):				
np01	34	8	-	231
np02	27	34	-	246
np03	25	10	-	130
np04	24	-	-	152
np05	22	42	-	129
np06	19	27	-	146
np08	25	38	-	171
np10	19	41	-	125
np11	19	37	-	149
np12	30	37	-	157
np13	22	8	-	101
np14	21	38	-	114
np16	30	34	-	91
np17	19	8	-	137
np18	23	34	-	183
np19	29	8	-	102
np20	16	-	-	116
np21	28	36	-	52
np22	20	36	-	152
np23	-	-	-	116
np24	20	39	-	-
np25	33	12	-	134
np26	20	-	-	146
np27	21	22	-	171
np28	21	9	-	131

Plasma 6-keto $PGF_1\alpha$ Values (continued)

patient #	age	gestational age	platelet count	plasma 6-keto $PGF_1\alpha$
np29	20	-	-	174
np30	21	10	-	117
np31	38	36	-	212
np32	23	36	-	237
np33	22	30	-	148
np34	19	21	-	164
np35	26	24	-	-
np36	25	32	-	-
np37	31	-	-	-
np38	19	35	-	-
np39	30	35	-	-
np40	19	37	-	217
np41****	19	32	-	193
np42	18	37	-	223
np43	21	36	-	280
np44	41	36	-	-
np45	19	36	-	348
np46	19	22	-	161

mean - 163
S.D. - 58

**** - twin gestation

*** - eclampsia

** - severe preeclampsia

* - chronic hypertension

Study II.

A. Assay Parameters

$\%B_o$ (Binding with no antigen) - 41.6%

Correlation of logit vs. ln (dose) r^2 - 0.961

Specificity Cross-reactivity is the same as in study I.

Nonspecific binding of blanks:

	liquid-liquid extraction	solid phase extraction	without extraction
stripped plasma	64	*	*
	*	-	196
	*	-	218
distilled water	450	27	*
	91	*	*
	94	295	*

Sensitivity

1) Mass for logit - 0 (mass needed to displace 50% of the labeled antibody) - 154.8 pg
2) "Least detectable mass" (mass resulting in a response two standard deviations from the response to a zero dose) - for liquid/liquid extraction, 211 - 2* 292 - 795 pg
   Not determinable for SPE or nonextracted blanks.

Study II.

Precision and Accuracy

Measured and actual amounts of 6-keto $PGF_1\alpha$ in serial solutions of plasma and water were compared:

Amount of 6-keto $PGF_1\alpha$ added to solvent	Measured values in samples treated by: ethyl acetate extraction	solid phase extraction	no extraction
solvent- charcoal-stripped plasma			
0 pg/ml	*	*	*
0	*	*	196
0	64	*	219
62	*	*	283
62	*	*	255
125	*	*	237
250	*	*	270
500	*	*	345
1000	55	-	339
2000	-	95	66
2000	-	-	4394
			r^2 - 0.205 y-intercept - 117 slope-0.64
solvent - distilled water			
0	450	27	*
0	91	295	*
0	94	-	*
62	95	80	159
62	120	-	150
125	*	50	127
250	15	161	490
500	254	161	490
1000	256	244	699
2000	359	175	1159
2000	-	-	3319
	r^2 - 0.239 y-intercept - 127 slope-0.11	r^2 - 0.235 y-intercept - 106 slope-0.06	r^2 - 0.738 y-intercept - -20 slope - 1.08

* - negative value
- - no data available

Study II.

Plasma 6-keto $PGF_1\alpha$ values:

patient	age	gestational age	platelet count	6-keto $PGF_1\alpha$ in: ethyl acetate	SPE	none
controls:						
50	35	9 weeks	-	55	-	117
51	19	25	230,000	301	-	-
52	18	-	-	102	-	-
53	19	16	-	116	22	53
54	26	18	-	151	-	48
55**	20	36	220,000	180	38	43
56	23	-	-	92	30	63
57	20	-	-	122	-	166
58	24	-	-	89	-	*
59	23	16	190,000	93	*	67
60	18	35	200,000	56	*	73
61	22	33	180,000	364	*	*
62	24	36	180,000	101	28	*
63	24	-	-	140	-	-
65	26	18	210,000	89	-	-
66	23	8	240,000	97	23	91
67	19	23	220,000	111	-	61
68	25	-	-	176	-	45
69	26	34	260,000	133	27	40
70	19	37	190,000	100	*	*
71	19	16	360,000	103	*	93
72	23	34	190,000	37	27	*
73	22	32	190,000	-	23	50
74	19	-	-	129	-	*
			mean	130	27	72
			S.D.	76	5	34

** twin gestation
* uninterpretable (negative) values
- no data available

Study II.

Plasma 6-keto $PGF_1\alpha$ values:

patient	age	gestational age		platelet count	6-keto $PGF_1\alpha$ in: ethyl acetate	SPE	none
preeclamptics:							
64	17	29		60,000	*	*	*
016	19	33		200,000	-	19	45
017	18	28		65,000	61	*	*
018	25	39		150,000	72	-	*
019	32	31		110,000	93	-	-
				mean -	75	S.D. - 16	
longitudinal study of a preeclamptic:							
015	33	31	1/24	140,000	129	-	66
			1/26	180,000	124	45	72
			1/27	210,000	-	10	*
			1/28	250,000	124	*	21
			1/29	250,000	*	*	90
			1/30	240,000	129	*	38
			1/31	240,000	130	37	53
			2/1	250,000	113	*	*
			2/4	-	105	27	41
			2/11	-	130	*	*

C. Correlation of 6-keto $PGF_1\alpha$ with Clinical Findings

In study I, the correlation between 6-keto $PGF_1\alpha$ and platelet count in preeclamptics ($r=0.73$) was found to be statistically significant, as was the correlation between 6-keto $PGF_1\alpha$ and gestational age in the controls ($r=0.285$, p 0.05) The difference in 6-keto $PGF_1\alpha$ in preeclamptics ($N=9$) and controls ($N=15$) in the twenty seventh week of gestation and beyond was not stasticically significant.

The correlation of 6-keto $PGF_1\alpha$ and systolic blood pressure, diastolic pressure, maternal weight, infant weight, and maternal age was not significant. 6-keto $PGF_1\alpha$ levels were not different between primiparas and multiparas in the control group. All preeclamptic women were primiparas except one with a history of preeclampsia in previous pregnancies.

1) <u>6-keto $PGF_1\alpha$ vs. platelet count in preeclamptics</u>

$r = 0.7333$ $N = 11$ $t = 3.235$ $0.05>p>0.01$

2) <u>6-keto $PGF_1\alpha$ vs. Gestational age in controls</u>

$r = 0.285$ $N = 31$ $t = 2.118$ $0.05>p>0.01$

3) <u>6-keto $PGF_1\alpha$ vs. gestational age in preeclamptics</u>

$r = 0.189$ $N = 12$ $t = 0.751$ N.S.

3) 6-keto PGF,α vs. systolic blood pressure in controls

r =-0.319 N = 30 t = 1.783 N.S.

4) 6-keto PGF,α vs. diastolic blood pressure in controls

r =-0.11889 N = 37 t = 0.749 N.S.

4) 6-keto PGF,α vs. maternal weight in controls

r = 0.859 N = 24 t = 0.787 N.S.

5) 6-keto PGF,α vs. infant birth weight in all

r = 0.277 N = 17 t = 1.074 N.S.

6) 6-keto PGF,α vs. maternal age in controls

r = 0.049 N = 35 t = 0.282 N.S.

7) 6-keto PGF,α in primiparous vs. multiparous controls

nulliparas N = 12, x = 165 pg/ml; multiparas N = 18, x = 155 pg/ml

S_{D_x} = 23.96 t = 0.004 N.S.

8) 6-keto PGF,α in preeclamptics vs. controls

preeclamptics N = 9, x = 149 pg/ml, S.D.= 70 pg/ml; controls N = 15, x = 191 pg/ml, S.D.= 73 pg/ml

S_{D_x} = 35.31 t = 1.189 N.S.

N.S. - not statistically significant

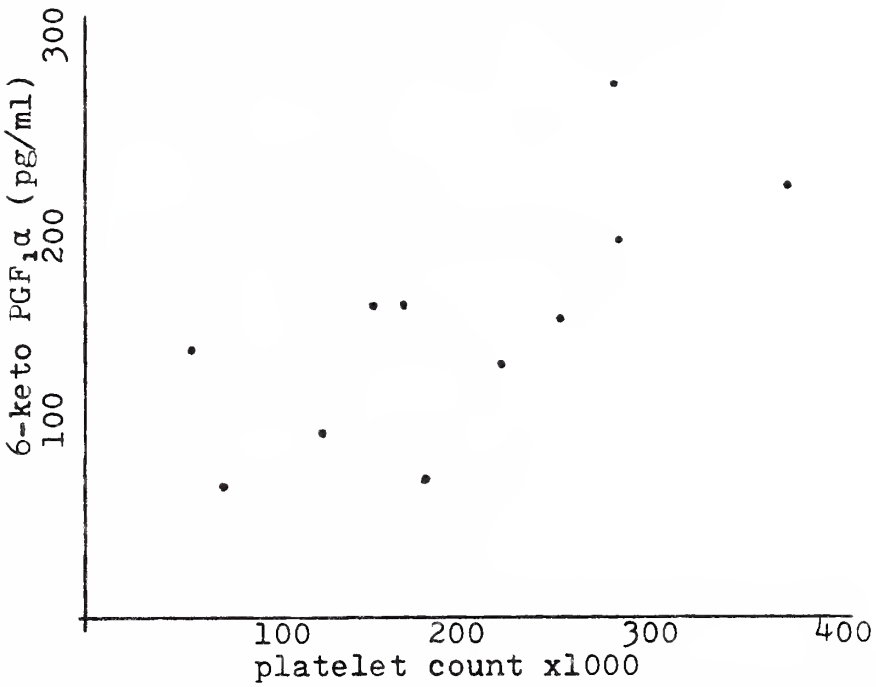


fig. 8. 6-keto PGF$_1\alpha$ vs. platelet count in preeclamptics

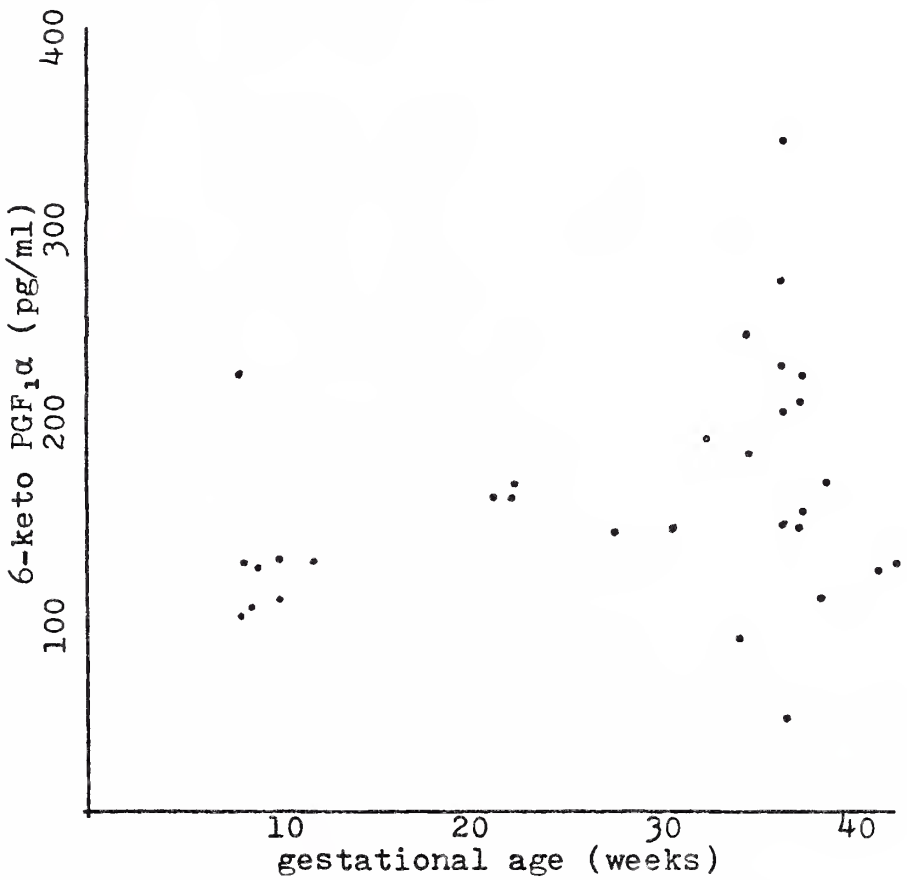


fig. 9. 6-keto PGF$_1\alpha$ vs. gestational age in controls

VII. Discussion

1) Validity of Data

The data from the two studies must be considered unreliable because of the inability to show appropriate results with the measurements of the serial dilutions of standards.

In study I, the extraction procedure appeared to introduce antibody-reactive material. Significant 6-keto-$PGF_1\alpha$ was measured in distilled water blanks that were assayed after extraction (90 pg/ml) as compared to water blanks that were assayed without extraction. (0 - 2 pg/ml) The solvents used in the extraction were each tested individually, and the low measured values could not totally account for the high water blank.

The results of measurements of serial dilutions of known amounts of 6-keto $PGF_1\alpha$ in both pooled plasma and distilled water were done to assess the accuracy and parallelism of the system. The standards added to water which were then extracted showed fairly good correlation between measured and actual values, despite a high blank (90 pg/ml.) The standards in pooled plasma that had been

extracted with ethyl acetate showed poor correlation with actual values. Throughout the dilution range (0-2000 pg/ml), the measured values were fairly constant (65-151 pg/ml). This could be due to several factors: i) The extraction may have broken down added 6-keto $PGF_1\alpha$ into antigenicly unreactive components, or ii) the ethyl acetate may not extract all of the unphysiologically high amounts of the 6-keto $PGF_1\alpha$ from the aqueous phase of the plasma.

All samples in Study I were extracted with ethyl acetate. In view of the poor results fron extracted standards in plasma, the data from Study I cannot be considered reliable.

As part of Study I, a set of plasma standards which had not been extracted was assayed. The correlation between measured and actual concentrations was much better (r^2= 0.98, slope - 0.57) than the correlations of the extracted plasma samples (r^2- 0.81, slope - 0.03). Because of the high blanks and poor results in the plasma that had been extracted compared to those without extraction, it became clear that the extraction process itself caused a significant lack of precision and accuracy.

Thus to determine which method of preparation leads to the most precise and accurate results, Study II was done. Each control, and most samples in this study, were prepared in three different ways - ethyl acetate extraction

(liquid/liquid phase), solid phase extraction (SPE), and no extraction.

Blank values in the second study varied greatly, from negative values to 450 pg/ml. Correlations between measured and actual values of standard dilutions for both ethyl acetate extraction and no extraction were much worse than the same experiments done in Study I. Correlations for standards treated with solid phase extraction were equally poor. For all three methods, many of the results were uninterpretable because the radioactivity of the bound antigen in the sample were higher than that of the zero tubes (those with no added sample.) This means that negative values for 6-keto-$PGF_1\alpha$ were obtained.

It is apparent that the assay itself did not work appropriately in Study II. The standard curve in buffer, used to determine the dose-response curve, was not acceptable, as shown below.

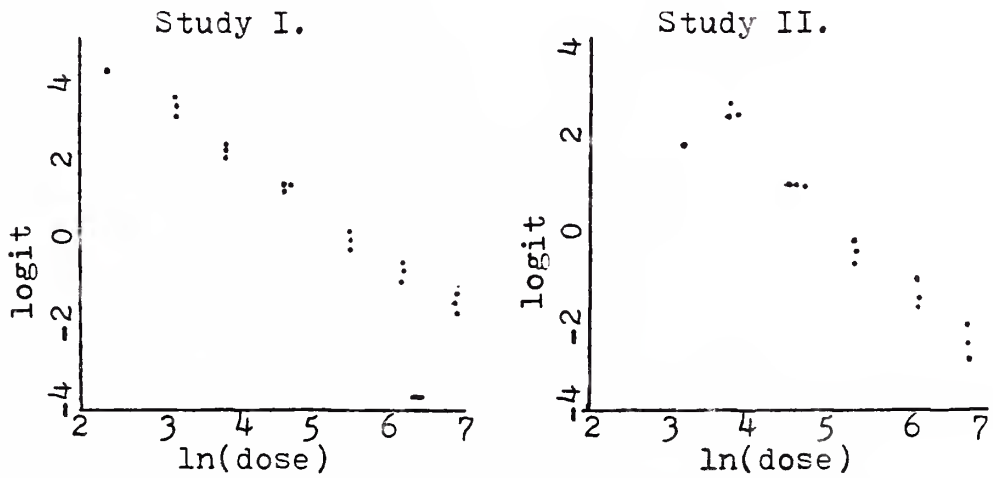


fig. 10. Dose-response curves for Studies I and II.

This failure of the assay in Study II could be due to several factors:

i) Error in experimental technique. Although conditions for the assay (treatment of reagents, pipetting technique, equipment, incubation period, and charcoaling technique) were kept as constant as possible, errors in performing the assay could not be ruled out.

ii) Siliconized test tubes. In study I, the test tubes for the assay were unsiliconized borosilicate glass. Test tubes for Study II were siliconized according to the NEN assay kit instructions. This was to decrease the amount of 6-keto $PGF_1\alpha$ that adsorbed to the glass. The siliconizing agent or the method used may have altered the assay.

iii) Reagent Instability. Reagents had been refrigerated at 4°C for an additional month for Study II. Although lyophylized reagents were not reconstituted until the evening that the assay was performed, it is possible that the reagents may have been unstable. The charcoal was supplied as a suspension in phosphate buffer, and was reported to be stable for two months. There was a six week period between obtaining the suspension and running the assay for Study II.

It was hoped that Study II could suggest which method of sample preparation led to the most accurate results, and would also provide more data on 6-keto $PGF_1\alpha$ in the plasma of normal and preeclamptic women. However, with the study itself being unreliable, conclusions cannot be drawn from this study.

2) Correlation of 6-keto $PGF_1\alpha$ with Clinical Results

It is tempting to accept the data on plasma 6-keto $PGF_1\alpha$ obtained from Study I, despite the problems with verifying the assay's validity. Although there was poor correlation between measured and actual values in plasma samples spiked with various amounts of synthetic 6-keto $PGF_1\alpha$, the correlation is fairly good for water spiked with 6-keto $PGF_1\alpha$. More interestingly, the values found in plasma agree with previously reported results. Mean levels of 6-keto $PGF_1\alpha$ were low in early pregnancy (135 pg/ml), increased in mid pregnancy (158 pg/ml), and further increased in late pregnancy with greatly increased scatter (215 pg/ml), as seen in figure 8. This is statistically significant, and similar to the patterns found by Lewis et al. (77) and Bolton et al. (81).

6-keto $PGF_1\alpha$ was found to be lower in preeclamptics (149 pg/ml) as compared to normal women during the twenty-seventh week of gestation or later (191 pg/ml), but this difference was not statistically significant. If the assay was valid, this data would significant to help support the theory that decreased peripheral levels of PGI_2 might be an operant mechanism in preeclampsia-eclampsia.

Another finding was the statisically significant ($p < 0.05$) correlation of 6-keto $PGF_1\alpha$ with platelet count in women with preeclampsia. This could be expained two ways:

i) A decrease in prostacyclin *in vivo* caused an inbalance in the PGI_2/TXA_2 ratio, leading to increased intravascular coagulation and platelet consumption, and therefore lowered platelet count.

ii) A cross-reacting substance was released from the platelets upon sample collection.

It is difficult to distinguish between these possibilities without verification by GC-MS.

The results of this study offers some data that would be tempting to accept as evidence that peripheral 6-keto $PGF_1\alpha$, and therefore, PGI_2, is increased in normal pregnancy and decreased in women with preeclampsia. This cannot be accepted with enthusiasm beause assay parameters were not shown to be valid. GC-MS measurement of a subset of the samples may show whether 6-keto $PGF_1\alpha$ measured by RIA was preoportional to the 6-keto $PGF_1\alpha$ in the sample. Such evidence would make the findings of this study significant in the study of peripheral PGI_2 in preeclampsia.

VIII. Summary

Prostacyclin production and activity in preeclampsia-eclampsia is currenly under investigation at several laboratories. Because of prostacyclin's various effects (platelet aggregation inhibition, vasodilation, stimulation of renin release, and possible regulator of the hypertensive effects of angiotensin II), an increase in the peripheral activity of this chemical would explain much of the altered physiology of normal pregnancy. A relative decrease in peripheral and local action could theoretically tie together the many pathophysiological findings of preeclampsia-eclampsia.

Therefore, plasma 6-keto $PGF_1\alpha$, the stable nonenzymatic metabolite of prostacyclin, was measured in women with preeclampsia and in those of various gestational ages having an uncomplicated pregnancy. The detection method, radioimmunoassay, could not be internally validated. It appears that the major problem with the assay is in sample preparation.

Plasma 6-keto $PGF_1\alpha$ may decrease in those women with preeclampsia compared to normals of the same gestational age, as shown in this study. An increase was seen throughout normal pregnancy. In addition, plasma 6-keto $PGF_1\alpha$ was seen

to correlate with platelet count in the preeclamptic women.

These results cannot be accepted as reliable, but instead should be considered as impetus for further study. Gas chromatography-mass spectrometry of some of the samples from the study could be used for verification. Platelet aggregation inhibition bioassay with confirmation by GC-MS, is probably the most reliable detection method available to date, and would be the best method for further study.

If PGI_2 activity and levels are truly decreased in preeclampsia, not only does the mechanism of the disease become much more clear, but a more acceptable treatment modality for the disease is suggested.

REFERENCES

1. Hughes, E.C., Obstetric-Gynecologic Terminology. Philadelphia: Davis,1972.

2. Jespersen, J. "Disseminated Intravascular Coagulation in Toxemia of Pregnancy. Correction of the Decreased Platelet Counts and Raised levels of Serum uric Acid and Fibrin Degredation products by Aspirin." Thrombosis Research, 1980, 17, 743-746.

3. Chesley, L.C. "False Steps in the Study of Preeclampsia." In Lindheimer,M.D., Katz, A.I., and Zustan, F.P.(Ed.), Hypertension in Pregnancy. New York: John Wiley and Sons, 1976.

4. Willson, C. " Hypertensive Disorders of Pregnancy." In Willson, C. (Ed.), Obstetrics and Gynecology. C.V. Mosby Co, 1979, 335-347.

5. Rafferty,T.D. & Berkowitz, R.L. "Hemodynamics in Patients with Severe Toxemia during Labor and Delivery." American Journal of Obstetrics and Gynecology, 1980, 138, 263-270.

6. Symonds, E.M. "Aetiology of Preeclampsia- a Review." Journal of the Royal Society of Medicine, 1980, 73, 871-875.

7. Chesley, L.C. "Plasma and Red Cell volumes during Pregnancy." American Journal of Obstetrics and Gynecology, 1972, 112, 440-450.

8. Chesley, L.C. Hypertensive disorders of Pregnancy. New York, Appleton-Century-Crofts, 1978, pp. 202-203.

9. Pritchard, J.A. "Management of Preeclampsia and Eclampsia." Kidney International, 1980, 18, 259-266.

10. Symonds, E.M. "The Renin-angiotensin System and Sodium excretion during Gestation." In Lindheimer, M.D., Katz,A.I., & Zustan, F.P. (ed.), Hypertension in Pregnancy. New York, John Wiley and Sons, 1976, pp.271-279.

11. Oparil, S., Low, J., Ehrlich, E., & Lindheimer, M. The Renin-Angiotensin System in Mother and Fetus at Cesarean Section: a Preliminary Communication." In Lindheimer, M.D., Katz, A.I., and Zustan, F.P. (Ed.), Hypertension in Pregnancy. New York: John Wiley and Sons, 1976, pp.287-292.

12. Ferris, T.F. "Postpartum Renal Insufficiency." Kidney International, 1978, 14, 383-392.

13. Gant, N., Chand, S.G., Cunningham, S., and MacDonald, P. "Control of Vascular Reactivity to Angiotensin II in Human Pregnancy." In Lindheimer, M.D., Katz, A.I., & Zustan, F.P.(Ed.), Hypertension in Pregnancy. New York: John Wiley and Sons, 1976, pp.377-389.

14. Gant, N.F., Daley, G.L., Chand, S., Whalley, P.J., MacDonald, P.C. "A study of Angiotensin II Pressor response throughout primigravid Pregnancy." Journal of Clinical Investigation, 1973, 52, 2682.

15. Pritchard, J.A. "Hypertensive Disorders of Pregnancy." In William's Obsttrics, 1980, pp.665-700.

16. Weir, R.J., Fraizer, R., Lever, A.F., Morton, J.J., Brown, J.J., Krawszewski, A., McIlwaine, G.M., Robertson, J.I.S, and Tree, M. "Plasma Renin, Renin Substrate, Angiotensin II, and Aldosterone in Hypertensive Disease of Pregnancy." The Lancet, 1973,291-294.

17. Weir, R.J., Doig, A., Fraizer, J.J., Morton, J.J., Parboosingh, J., Robertson, J.I.S., & Wilson, A. "Studies of the Renin-Angiotensin-Aldosterone System, Cortisol, DOC, and ADH in Normal and Hypertensive Pregnancy." In Lindheimer, M.D., Katz, A.I., & Zuspan, F.P. (Ed.), Hypertension in Pregnancy. New York: John Wiley and Sons, 1976,pp.251-261.

18. Weinberger, M.H., Kramer, N.J., Peterson, L.P., Cleary, R.E., & Young, P.C. "Sequential Changes in the Renin-Angiotensin-Aldosterone Systems and Plasma Progesterone Concentration in Normal and Abnormal Human Pregnancy." In Lindheimer, M.D., Katz, A.I., and Zuspan, F.P. Hypertension in Pregnancy. New York: John Wiley and Sons, 1976, pp. 263-269.

19. Satoh, H., Matsukura, K., Yamanda, R., Satoh, S. "Influence of Angiotensins (I,II,III) on Prostaglandin Production by minced Rat Medulla." Prostaglandins, 1981, 21, 973-984.

20. Gerber, J.G., Branch, R.A., Nies, A.S., Gerkins, J.F., Shand, D.G., Hollifield, & Oates, J.A. "Prostaglandins and Renin Release: Assessment of Renin Secretion following Infusion of PFI_2, PGE_2, and PGD_2 into the Renal Artery of Anesthetized Dogs." Prostaglandins, 1978, 15, 81-88.

21. Ganong, W.F. Review of Medical Physiology. Los Altos, California: Lange Medical Publications, 1977, pp. 346-347.

22. Vallotton, M.B., Godard, C., & Gaillard, R. "Assessment of the Renin-Angiotensin-Aldosterone System, aldosterone, and Cortisol in Mother and Fetus at Term." In Lindheimer, M.D., Katz, A.I., and Zuspan, F.P. (Ed.), Hypertension in Pregnancy. New York: John Wiley and Sons, 1976, pp.281-286.

23. Everett, R.B., Worley, R.J., MacDonald, P.C., & Gant, N.F. "Effect of Prostaglandin Synthetase Inhibitors on Pressor REsponse to Angiotensin II in Human Pregnancy." Journal of Clinical Endocrinology and Metabolism, 1978, 46,1007.

24. Gant, N.F., Worley, R.J., MacDonald, P.C. "The Clearance Rate of Maternal Plasma Prehormones of Placental Estrogen Formation." In Lindheimer, M.D., Katz, A.I., & Zuspan, F.P. (Ed.), Hypertension in Pregnancy. New York: John Wiley and Sons, 1976, pp. 309,314.

25. Gerretsen, G., & Huisjes, H.J. "Morphological Changes of the Spiral Arteries in the Placental Bed in Relation to Preeclampsia and Fetal Growth Retardation." Brittish Journal of Obstetrics and Gynecology, 1981, 88, 876-881.

26. Bonnar, J., & Sheppard, B.L. "Coagulation Activation and Local Vascular Changes in Preeclampsia." Contributions to Nephrology, 1981, 25, 98-107.

27. Robertson, W.B., Brosens, I., & Dixon, G. "Maternal Uterine Vascular Lesions in the Hypertensive Complications of Pregnancy." In Lindheimer, M.D., Katz, A.I., & Zuspan, F.P. Hypertension in Pregnancy. New York: John Wiley and Sons, 1976, pp.115-127.

28. Kuhn, W., & Graeff, H. "Coagulation Problems in Pregnancy." Contributions to Nephrology, 1981,25, pp.78-84.

29. McKay, D.G. "Chronic Intravascular Coagulation in Normal Pregnancy and Preeclampsia." Contributions to Nephrology, 1981, 25, pp.108-119.

30. Howie, P.W., Begg, C.B., Purdie, D.W., & Prentice, C.R.M. "Use of Coagulation Tests to Predict the Clinical Progress of Preeclampsia." The Lancet,1976, 323-325.

31. Rákóczi, I., Tallián, F., Bagdány, S. & Gàti, I. "Platelet Life Span in Normal Pregnancy and Preeclampsia as Determined by a Non-radioactive Technique." Thrombosis Research, 1979, 15, 553-556.

32. Whigham, K.A., Howie, P.W., Drummond, A.H., & Prentice, C.R.M. "Abnormal Platelet Function in Preeclampsia." Brittish Journal of Obstetrics and Gynecology, 1978, 85,pp.28-32.

33. Robson, J.S. "Proteinuria and the Renal Lesion in Preeclampsia and Abruptio Placentae." In Lindheimer, M.D., Katz, A.I., & Zuspan, F.P. (Ed.), Hypertension in Pregnancy. New York: John Wiley and Sons, 1976, pp. 61-73.

34. Lison, A.E., and Marx, M. "Proteinuria in Relation to Toxemia of Pregnancy." Contributions to Nephrology, 1981,25, 128-131.

35. Barthels, M. & Reidel, H. "Fibrin(ogen) Degrdation Products in Normal Pregnancy and Toxaemia of Pregnancy." Contributions to Nephrology, 1981, 25, 85-89.

36. Spargo, B.H., Lichtig, C., Luger, A.M., Katz, A.I., and Lindheimer, M.D. "The Renal Lesion in Preeclampsia." In Lindheimer, M.D., Katz, A.I., and Zuspan, F.P. Hypertension in Pregnancy. New York: John Wiley and Sons, 1976, pp.129-136.

37. Sheehan, H.L. "Renal Morphology in Preeclampsia." Kidney International, 1980,18, 241-252.

38. Dass, A., & Bhagwanani, S. "Serum Transaminases in Toxemia of Pregnancy." Journal of Obstetrics and Gynecology, 1961, 727-734.

39. Gedekoh, R.H., Hayashi, T.T., & MacDonald, H.M. "Eclampsia at McGee Women's Hospital, 1970-1980." American Journal of Obstetrics and Gynecology,1981,140,860-866.

40. MacGillivray,I., and Campbell, D.M. "A Prospective Study of Factors Affecting Intrauterine Growth." In Lindheimer, M.D., Katz, A.I., & Zuspan, F.P. (Ed.), Hypertension in Pregnancy. New York: John Wiley and Sons, 1976, pp.23-29.

41. Wood, S.M., Burnett, D., & Studd, J. "Selectivity of Proetinuria During Pregnancy Assessed by Different Methods." In Lindheimer, M.D., Katz, A.I., & Zuspan, F.P. Hypertension in Pregnancy. New York: John Wiley and Sons, 1976, pp. 75 83.

42. Beherman, H.R. "Chemistry, Metabolism, and Pharmacology of Prostaglandins." Lecture, Yale Medical School, October L979.

43. McGiff, J.C. "Prostaglandins, Prostacyclin, and Thromboxanes." Annual Review of Pharmacology and Toxicology, 1981, 21,479-509.

44. Hall, A.K., & Behrman, H.R. "Prostaglandins: Biosynthesis, Metabolism, and Mechanism of Cellular Action." In Lee, J.B. (Ed.) Prostaglandins. New York; Elsevier, 1982, pp.1-38.

45. Moncada, S., and Vane, J.R. "Arachidonic Acid Metabolites and the Interactions between Platelets and Blood Vessel Walls." New England Journal of Medicine, 1979, 300,1142-1147.

46. Allan,G., & Eakins, K.E. "Burimamide is a Selective Inhibitor of Thromboxane-A Synthesis in Human Platelet Microsomes." Prostaglandins, 15, 1978, 659-661.

47. Hamberg, M., Svensson, J., & Samulsson, B. "Thromboxanes: A New Group of Biologically Active Compounds Derived from Prostaglandin Endoperoxides." National Academy of Science- Proceddings, 1975, 72, 2994-2998.

48. Anderson, M.W., Crutchley, D.J., Tainer, B.E., & Eling, T.E. "Kinetic Studies on the Conversion of Prostaglandin Endoperoxide PGH_2 by Thromboxane Synthetase." Prostaglandins,1978, 16, 563-570.

49. Masotti, G., Poggessi, L., Galanti, G., Abbate, R., & Neri Serneri, G.G. "Differential Inhibition of Prostacyclin Production and Platelet Aggregation by Aspirin." The Lancet,1979, 1213-1216.

50. Moncada, S., Gryglewski, R.J., Bunting, S., & Vane, J.R. "A Lipid Peroxide that Inhibits the enzyme from the Blood Vessel Microsomes that generates from Prostaglandin Endoperoxides the Substance (Prostaglandin X) which Prevents Platelet Aggregation." Prostaglandins,1976, 12, 715-735.

51. Johnson, R.A., Morton, D.R., Kinner, J.H., Gorman, R.R., McGuire, J.C., Sun, F.F., Whittacker, N., Bunting, S., Salman, J., Moncada, S. & Vane, J.R. "The Chemical Structure of Prostaglandin X (Prostacyclin). Prostaglandins,1976, 12, 915-928.

52. Weksler, B.B., Marcus, A.J., & Jaffe, E.A. "Synthesis of Prostaglandin I_2 (Prostacyclin) by Cultured Human and Bovine Endothelial Cells." Proceedings of the National Academy of Science,1977, 74,3922-3926.

53. MacIntyre, D.E., Pearson, J.D., & Gordon, J.L. "Localization and Stimulation of Prostacyclin in Vascular Cells." Nature, 271,1978, 549-551.

54. Wong, P.Y-K., Lee, W.H., Chao, P. H-W., Reiss, R.F., & McGiff, J.C. "Metabolism of Prostacyclin by 9-Hydroxyprostaglandin dehydrogenase in Hman Platelets." The Journal of Biological Chemistry, 255,1980, 9020-9024.

55. Elebute, O.A., & Mills, I.H. "Urinary Kallikrein in Normal and Hypertensive Pregnancies." In Lindheimer, M.D., Katz, A.I., & Zuspan, F.P. Hypertension in Pregnancy. New York: John Wiley and Sons, 1976, pp.329-338.

56. Miyamori, I., FitzGerald, G.A., Brown, M.J., & Lewis, P.J. "Prostacyclin Stimulates the Renin Angiotensin Aldosterone System in Man." Journal of Clinical Endocrinology and Metabolism, 1979,49, 943-945.

57. Sperroff, L., Hanning, R.V., Ewaschuk, E.J., Alberino, S.L., & Kieliszek, F.X. "Uterine Atery Blood Flow Studies in the Pregnant Monkey." In Lindheimer, M.D., Katz, A.I., & Zuspan, F.P. Hypertension in Pregnancy, New York: John Wiley and Sons, 1976, pp. 315-327.

58. Everett, B.B., Worley, R.J., MacDonald, P.C., Gant, N.F. "Effect of Prostaglandin Synthetae Inhibitors on Pressor Response to Angiotensin II in Human Pregnancy." Journal of Clinical Endocrinology and Metabolism, 1978, 46, 1007-1010.

59. Demers, L.M., and Gabbe, S.G. "Placental Prostaglandin Levels in Preeclampsia." American Journal of Obstetrics and Gynecology, 1978, 126, 137-139.

60. Terragno, N.A., McGiff, J.C., Smigel, M., & Terragno, A. "Patterns of Prostaglandin Production in the Bovine Fetal and Maternal Vasculature." Prostaglandins, 1978,16, 847-856.

61. Pifer, D., Cagen, L.M., Chesney, C.M.cI. "Stability of Prostacyclin in Human Blood." Prostaglandins, 21, 1981, 165-175.

62. McGiff, J.C., Crowshaw, K., Terragno, N.A., Lonigro, A.J. "Release of a Prostaglandin-like Substance into Renal venous blood in Response to Angiotensin II." Circulation Research, 31-32, Suppliment 1, pp.121-130.

63. Speroff, L. "An essay: Prostaglandins and Toxemia of Pregnancy." Prostaglandins, 1973, 3, 721-728.

64. Skrinska, V. & Lucas, F.V. "Isolation of Prostacyclin from Whole Blood." Prostaglandins,1981, 22, 365-375.

65. Speroff, L. "An Autoregulatory Role for Prostaglandins in Placental Hemodynamics: Their Possible influence on Blood Pressure in Pregnancy." The Journal of Reproductive Medicine, 1975, 15, 181-188.

66. Speroff, L., Haning, R.V., & Levin, R.M. "The effect of Angiotensin II and Indomethacin on Uterine Artery Blood Flow in Pregnant Monkeys." Obstetrics and Gynecology, 1977, 50,611-614.

67. Rankin, J.H.G., Berssenbrugge, A., Anderson, D., Phernetton, T. "Ovine Placental Vascular Responses toIndomethacin." American Journal of Physiology, 1979, H61-H64.

68. Terragno, N.A., Terragno, D.A., McGiff, J.C. "The Role of Prostaglandins in the Control of Uterine Blood Flow." In Lindheimer, M.D., Katz, A.I., & Zuspan, F.P. (Ed.) Hypertension in Pregnancy. New York: John Wiley and Sons, 1976, pp.391-398.

69. Bygdeman, M., & Gillespie, A. "Prostaglandins in Human Reproduction." In Lee, J.B. (Ed.) Prostaglandins. New York: Elsevier, 1982, pp.215-249.

70. Mitchell, M.D., Robinson, J.S.,.Redman, C.G.W., & Clover, L. "The concentrations of Prostaglandins E and F, 13,14-dihydro-15-oxo-prostaglandin F and Thromboxane B_2 in tissues Obtined from Women with and Without Preeclampsia." Prostaglandins and Medicine, 1979, 3, 223-234.

71. Novy, and Liggins "The Role of Prostaglandins in Parturition." Seminars in Perinatology, 1980, 4, 45-65.

72. Valenzuela, G., & Bodkhe, R.R. "Effect of Pregnancy-Induced Hypertension on Placental Prostaglandin Metabolism: Decreased $PGF_2\alpha$ catabolism with normal PGE_2 catabolism." American Journal of Obstetrics and Gynecology, 1980, 255-256.

73. Mitchell, M.D., Bibby, J.G., Hicks, B.R., Redman, C.W.G., Anderson, A.B.M., & Turnbull, A.C. "Thromboxane B_2 and Human Parturition: Concentrations in the Plasma and Production in vitro." Journal of Endocrinology, 1978, 78,435-441.

74. Mitchell, M.D., Bibby, J.G., Hicks, B.R., & Turnbull, A.C. "Possible Role for Prostacyclin in Human Parturition." Prostaglandins,1978,16, 931-937.

75. O'Brien, P.M.S., Broughton Pipkin, E. "The effect of Deprivation of Prostaglandin Precursors on Vascular Sensitivity to Angiotensin II and on the Kidney in the pregnant rabbit. Brittish Journal of Pharmacology, 65,1978,29-34.

76. Turner, G., & Collins, E. "Fetal Effects of Regular Salicylate Ingestion in Pregnancy." The Lancet, 1975, 338-339.

77. Lewis, P.J., Boylan, P.,Friedman, L.A., Hensby, C.N., Downing, I. "Prostacyclin in Pregnancy." Brittish Medical Journal, 1980, 1581-1582.

78. Hamberg, M., Tuvemo, T., Svensson, J., & Jonsson, C-E. "Formation and Action of Prostacyclin in the Isolated Human Umbilical Artery." Acta Physiologia Scandinavia, 1979, 106,289-292.

79. Remuzzi, G., Misiani, R., Muratore, D., Marchesi, D., Livio, M., Schieppati, Mecca, G., Gaetano, G., Donati, M.B. "Prostacyclin and Human Fetal Circulation." Prostaglandins, 1979, 18, 341-348.

80. Tanaka, M., Morita, I., Hirakawa, S., Murota, S. "Increased Prostacyclin Synthesizing Activity in Human Ripening Cervix." Prostaglandins, 1981, 21, 83-86.

81. Bolton,P.J., Jogee, M., Myatt, L., & Elder, M.G. "Maternal 6-oxo-Prostaglandin $F_1\alpha$ levels throughout Pregnancy: A Longitudinal Study." Brittish Journal of Obstetrics and Gynecology, 1981, 88, 1101-1103.

82. Ylikorkala, O., & Vinikka, L. "Maternal Plasma 6-keto Prostaglandin $F_{1\alpha}$ during Pregnancy and Puerperium." Prostaglandins and Medicine, 7,1981, 95-99.

83. Remuzzi, G., Marchesi, D., Mecca, G., Misiani, R., Rossi, E., Donati, M.B., & Getano, G. "Reduction of Fetal Vascular Prostacyclin Activity in Preeclampsia." The Lancet, 1980, 310.

84. Remuzzi, G., Marchesi, D., Zoja, C., Muratore, D., Mecca, G., Misiani, R., Rossi, E., Barbato, M., Capetta, P., Donati, M., and Gaetano, G. "Reduced Umbilical Placental Vascular Prostacyclin in Severe Preeclampsia." Prostaglandins, 1980, 20, 105-110.

85. Downing, I., Shepard, G.L., & Lewis, J.F. "Redused Prostacyclin Prodution in Preeclampsia." The Lancet, 1980, 1374.

86. Page, E.W., "On the Pathogenesis of Preeclampsia and Eclampsia." The Journal of Obstetrics and Gynecology, 1972, 79, 883-894.

87. Carreras. L.O., Defreyn, G., Van Houtie, E., Vermylen, J., Van Assche, A. "Prostacyclin and Preeclampsia." The Lancet, 1981, 442.

88. Stuart, M.J., Sunderi, S.G., Yambo, T., Clark, D.A., Allen, J.B., Elrad, H.,& Slott, J.H. "Decreased Prostacycin Production: A Characteristic of Chronic Placental Insufficiency Syndromes." The Lancet, 1981, 1126-1128.

89. Bodzenta, A., Thompson, J.M., Poller, L. "Prostacyclin Activity in Amniotic Fluid in Preeclampsia." The Lancet, 1980, 650.

90. Ylikorkala, O., Makila, U.-M., & Viinikka, L. "Amniotic Fluid Prostacyclin and Thromboxane in Normal, Preeclamptic, and some other Complicated Pregnancies." American Journal of Obstetrics and Gynecology, 141, 1981, 487-490.

91. Lewis, P.J., Shepard, G.L., Ritter, J., Chan, S.M.T., Bolton, P.J., Jogee, M., Myatt, L., & Elder, M.G. "Prostacyclin and Preeclampsia." The Lancet, 1981, 559.

92. Fidler, J., Bennett, M.J., deSwiet, M., Ellis, C., Lewis, P.J. "Treatment of Pregnancy Hypertension with Prostacyclin." The Lancet, 31-32.

93. Remuzzi, G., Zoja, C., Marchesi, D., Scheppati, A., Mecca, G., Misiani, R., Donati, M.B., & Gaetano, G. "Plasmatic Regulation of Vascular Prostacyclin in Pregnancy." Brittish Medical Journal, 1981, 282, 512-514.

94. Ylikorkala, O, Kikinen, P., & Viinikka, L. "Maternal Plasma Prostacyclin Concentration in Preeclampsia and Other Pregnancy Complications." Brittish Journal of Obstetrics and Gynecology, 1981, 88, 968-972.

95. Gaetano, G., Miragliotta, G., Roncucci, R., Lansen, J., Lambelin, G. "Suloctidil : A Novel Inhibitor of Platelet Aggregation in Human Beeings." Thrombosis Research, 1976, 8, 361-371.

96. Granstrom, E., & Kindahl, H. "Radioimmunoassay of Prostaglandins and Thromboxanes." In Frolich, J.C. (Ed.), Advances in Prostaglandin and Thromboxane Research, volume 5. New York: Raven Press, 1978., pp. 119-210.

97. Ylikorkala, O., & Viinikka, L. "Measurement of 6-keto Prostaglandin $F_1\alpha$ in Human Plasma with Radioimmunoassay: Effect of Prostacyclin Infusion." Prostaglandins in Medicine, 1981, 6, 427-436.

98. Hensby, C.N., Jogee, M., Elder, M.G., & Myatt, L. "A comparison of the Quantitative Analysis of 6-keto $PGF_1\alpha$ in Biological Fluids by Gas Chromatography Mass Spectrometry and Radioimmunoassay." "Biomedical Mass Spectrometry, 1981, 8, 111-117.

99. Mitchell, M.D., Brennecke, S.P., Denning-Kendall, P.A., MacDonald Gibson, W.J., Saeed, S.A., & Collier, O.J. "Comparisons Between the Abilities of the Various Human and Ovine Plasmas to Inhibit Prostaglandin Synthesis." Prostaglandins and Medicine, 1981, 6, 495-501.

100. Morris, H.G., Sherman, N.A., Shepperdson, F.T. "Variablrs in the Radioimmunassay of Prostaglndins in Plasma." Prostaglandins,21, 1981, 771-788.

101. Jaffe, B.M., and Behrman, H.R. "Prostaglandins and Prostaglandin Metabolites." In Methods of Hormone Radioimmunoassay, 1979 (second edition) Academic Press, pp. 19- 43.

102. Dray, F. "Sensitivity and Specificity of Prostanoic Derivaties Radioimmunoassays: New Approach." Proceedings pp. 1-16.

103. Hall, E. Presbyterian-St. Luke's Medical Center, Chicago, Illinois. Personal Communication; February 4,1981.

104. Kihndahl, H., and Grandstrom, E. "Limitations in Measurements of Prostaglandins and Thromboxanes." Proceedings, pp. 17-43.

105. Bussolini, F., Benedetti, C., Massorio, M., Camussi, G. "Maternal Vascular Prostacyclin Activity in Preeclampsia." The Lancet, 1980, 702.

106. Mitchell, M.D. University of Texas-Southwestern; Dallas, Texas. Personal Communication: February 5,1981.

107. Hanning, R.V., Kieliszek, F.X., Alberino, S.P., and Speroff, L. "A Radioimmunoassay for 13,14-dihydro-15-keto Prostaglandin $F_2\alpha$ with Chromatography and Internal Recovery Standard." (unpublished.)

108. Feldman, H. & Rodbard, D. "Mathematical Theory of Radioimmunoassay." In O'Dell, W.D. & Daughaday, W.H. (Ed.), Principles of Competitive Protein-Binding Assays. Philadelphia: Lippincott Co., 1971, pp. 158-203.

109. Kolstoe, R.H. Introduction to Statistics for the Behavioral Sciences. Homewood, Illinois: The Dorsey Press, 1973. p. 338.

110. Ylorikala, O., Makarainen, L., & Viinikka, L. "Prostacyclin Production increases During Human Parturition." Brittish Journal of Obstetrics and Gynecology, 88,1981, 513-516.

YALE MEDICAL LIBRARY

Manuscript Theses

Unpublished theses submitted for the Master's and Doctor's degrees and deposited in the Yale Medical Library are to be used only with due regard to the rights of the authors. Bibliographical references may be noted, but passages must not be copied without permission of the authors, and without proper credit being given in subsequent written or published work.

This thesis by has been used by the following persons, whose signatures attest their acceptance of the above restrictions.

NAME AND ADDRESS	DATE